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Public Health and Marine-Hospital Service of the United States.

HYGIENIC LABORATORY.—BULLETIN No. 44.-48
MAY, 1908.

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REPORT No. 2

ON

THE ORIGIN AND PREVALENCE OF TYPHOID FEVER IN THE DISTRICT OF COLUMBIA.

(1907.)

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BY

M. J. ROSENAU, L. L. LUMSDEN,
and JOSEPH H. KASTLE.

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INTRODUCTION

This bulletin gives the results of our studies on typhoid fever in the District of Columbia during 1907. Our first report on this subject, for the year 1906, is contained in Hygienic Laboratory Bulletin No. 35.

This year our studies included epidemiological investigations of the disease during the typhoid fever season, daily bacteriological examinations of the raw and filtered Potomac River water, bacteriological and chemical examinations of many hundreds of samples of the Washington milk supply, laboratory tests to assist in the diagnosis of the disease, and examinations of specimens in a search for bacillus-carriers, etc.

Thus far our studies indicate that typhoid fever will cease to be a "problem" in any community having clean water, an uninfected milk supply, and in which cases of the disease are treated as dangerous and contagious.

Certain improvements in the situation should be noted. The shallow wells have been closed, and during the summer of 1907 filtered water of good sanitary quality was supplied the city of Washington. Bills have been introduced into Congress with the object of improving the milk supply. The sewerage system has been practically completed. Laws are being enforced for the condemnation of insanitary dwellings, and to effect other improvements in the general sanitary conditions.

The case and death rates for typhoid fever in the District of Columbia in 1907 were lower than recorded for any previous year.

The improvement in the typhoid fever situation within certain limits should go on from year to year. The price of such improvement, however, will be well directed and unceasing efforts on the part of the local authorities, with the cooperation of the medical profession. The water must remain of good quality, the market milk must be improved, and a greater regard must be had for the contagious nature of the disease.

Attention is called to certain modifications in chart No. 4 of Bulletin No. 35, rendered necessary by a reexamination of the records of the health office. Chart No. 3 in this report is based upon records furnished by the health officer and gives the corrected curve of the seasonal prevalence of typhoid fever in previous years.

We have again profited by the cooperation of Dr. W. C. Woodward, the health officer of the District of Columbia, and also the officers of the filtration plant. We are indebted to the hospitals throughout the city and to many physicians for help in the epidemiological studies.

In drawing up the conclusions and recommendations of this report we have had the benefit of consultation with the advisory board of the Hygienic Laboratory, composed of eminent scientists and sanitarians. This privilege is appreciated, and we desire to acknowledge the help we have received from the members of the board collectively and individually.

M. J. Rosenau,
Director Hygienic Laboratory, Chairman.
L. L. Lumsden,
Passed Assistant Surgeon.
Joseph H. Kastle,
Chief of Division of Chemistry, Recorder,
United States Public Health and Marine-Hospital Service.

REPORT NO. 2 ON THE ORIGIN AND PREVALENCE OF TYPHOID FEVER IN THE DISTRICT OF COLUMBIA.

(1907.)

Our study of the prevalence of typhoid fever in the District of Columbia was resumed in May, 1907, and all cases reported to the health office from May 1 to November 1 have been investigated. Six hundred and seventy-five cases were reported in this period. In regard to five of these cases no accurate information could be obtained, so they were abandoned, leaving 670 cases for consideration in this report.

DIAGNOSIS.

It was not practicable for us to study the cases in clinical detail, but judging by the histories obtained in some instances, we are of the opinion that quite a number of the cases were incorrectly diagnosed and should not have been placed on the records as cases of typhoid fever. However, as the percentage of error in diagnosis was probably no greater this year than in previous years, and in order that our figures for this year should be comparable to those for previous years, we have included in our epidemiological studies every case reported as typhoid fever by the attending physician.

With a view to forming some idea of the amount of error in diagnosis, we examined the blood of 27 cases reported as typhoid fever between March 4 and December 2, 1907. Five cubic centimeters of blood were withdrawn from an arm vein and planted at once into 10 c. c. of sterilized ox bile. This was incubated for 24 hours, and subcultures made upon Endo and other media. This part of the work was done for us by Passed Assistant Surgeon A. M. Stimson, in the Division of Pathology and Bacteriology of the Hygienic Laboratory.

The following is a summary of the results:

Number of specimens examined	27
Specimens in which *B. typhosus* was found	8
Specimens in which *B. paratyphosus* A was found	1
Total	9 = 33.3 per cent.

Cases from which blood was taken in first week	7
Positive	2 = 28. 5 per cent.
Cases from which blood was taken in second week	8
Positive	2 = 25. 0 per cent.
Cases from which blood was taken in third week	4
Positive	3 = 75. 0 per cent.
Cases from which blood was taken in fourth week	3
Positive	2 = 66. 6 per cent,
Cases in which the time of disease was not stated	5
Positive	0 = 0. 0 per cent.

Of the 27 cases examined probably a definite proportion were not typhoid at all, for many of these tests were made at the request of physicians in doubtful cases to clear up difficult diagnoses. Three of the cases, after being reported, were determined clinically not to have been typhoid fever. Further, it was not practicable to make more than one examination from each case. We now know that 5 c. c. of blood is not always sufficient to demonstrate the presence of the typhoid bacillus at a single examination. In one of our cases 10 c. c. of blood was taken and divided into two portions. One portion remained sterile, while the other gave a pure culture of the typhoid bacillus.

In each instance the identity of the bacillus was determined by complete cultural and agglutinative tests.

Excluding the three cases clinically determined not to have been typhoid, the percentage of instances in which the bacillus was recovered is brought up to 37.5 per cent. Judging by the results of other investigators, the method followed in making these blood examinations should have demonstrated the typhoid bacillus in a larger percentage of instances if all of the 27 cases had been typhoid fever.

The number of cases of typhoid fever in the District of Columbia which are unrecognized and not reported probably exceeds considerably the number reported as typhoid fever under mistaken diagnosis. Thus, error in diagnosis alone makes it impossible by epidemiological investigations to account for a considerable number of cases. All data obtained in regard to cases reported as typhoid fever but which are not are misleading and so obscure the general problem. Spread of infection directly by contact or indirectly by persons, flies, milk, etc., from cases unrecognized and unreported cannot be determined, especially in a large community.

From the standpoint of prophylaxis, the unrecognized cases are, of course, more important than the cases erroneously reported as typhoid fever. In order that the actual prevalence of typhoid fever in a community may be determined and that proper measures to prevent the spread of the disease may be carried out, the correct and early diagnosis of all cases is of fundamental importance.

TYPHOID FEVER CASE CARD.

In our study of the cases the following form was used, and all facts called for by this form were carefully investigated for each case. This form varies somewhat from that used last year.

Public Health and Marine-Hospital Service—Hygienic Laboratory.

TYPHOID FEVER CASE CARD.

Date of investigation, ———. Case No. ——.
Name, ———.
Age, ——. Color, ———. Sex, ———. Nationality, ———.
Probable date of onset, ———. Date definite symptoms, ———.
Name and address of physician:

RESIDENCE.

Resident of District of Columbia, —— years, —— months.
Residence when taken sick, ———; from ——— to ———.
Previous residences, ———; from ——— to ———.
Subsequent residence, ———; from ——— to ———.
Temporary absences from District of Columbia within 30 days prior ———.
Number of occupants, ———. Ages, ———.
Number of occupants who have had typhoid, ——. When, ———.
Newcomers in house within three months prior, ——.
Newcomers in house had typhoid? ———.
Servants, ——
- White—
  - Resident, ——. Typhoid? ———.
  - Nonresident, ——. Typhoid? ———.
- Colored—
  - Resident, ——. Typhoid? ———.
  - Nonresident, ——. Typhoid? ———.

Typhoid at homes of servants, ———. When? ———.
Disposal of sewage, ———. Water-closets in house? ——.
Water-closets in yard? ——. Privy? ——. Location, ———.
General sanitary condition of residence, ———.

OCCUPATION.

Place, ———; from ——— to ———.
Other cases:

WATER WITHIN THIRTY DAYS PRIOR.

——— solely; ——— principally.
——— occasionally.

FOOD WITHIN THIRTY DAYS.

Where taken, ———.
Milk (how used), ———; from ———.
Boiled? ———. Pasteurized? ———.
Ice cream? ———. Where? ———.
Uncooked fruits and vegetables, ———.
Shellfish, ———.

CONTACTS.

Association 30 days prior with patients in febrile stage, ———.
Association with suspected cases, ———.
Association with persons who have had typhoid within 6 months, ———; 1 year, ———; 2 years, ———; 3 years, ———; 4 years, ———; 5 years, ———.
Association 30 days prior with persons in contact with patients in febrile stage, ———.
Treatment of stools and urine of patients, ———.
Other precautions, ———.
Remarks: ———.
Summary: ———.

——— ———.
(Signature of investigator.)

IMPORTED CASES.

In determining the number of cases in which the infection was contracted out of the District of Columbia the same method, but with a little more detail, was adopted as in our study of the cases reported from June 1 to November 1, 1906, and as published in our previous report.[a]

The cases fall under the following heads:

	Cases.
(*a*) Infection undoubtedly contracted out of the District of Columbia	117
(*b*) Infection probably contracted out of the District of Columbia	30
(*c*) Infection contracted in or out of the District of Columbia, chances about equal	40
(*d*) Infection undoubtedly contracted in the District of Columbia	363
(*e*) Infection probably contracted in the District of Columbia	120
Total	670

Considering the cases on the percentage of chances of the infection having been contracted out of the District of Columbia, they are as follows:

	Cases.
(*a*) 117 cases, chances 100 per cent	117
(*b*) 30 cases, chances 75 per cent	22
(*c*) 40 cases, chances 50 per cent	20
(*d*) 363 cases, chances 0 per cent	0
(*e*) 120 cases, chances 12.5 per cent	15
670 cases. Total	174

On this estimate, about 26 per cent of the cases investigated contracted the infection out of the District of Columbia, as against about 15 per cent of the cases studied by us in 1906.

In estimating the chances of contracting the infection out of the District, the time element was given the greatest consideration, but the prevalence of typhoid fever in the communities visited and known exposure of the person to infection while away were also taken into account. It should be borne in mind that the number of imported cases in a city is probably wholly or partly offset by the number of cases which contract the infection in the city and develop the disease elsewhere.

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[a] Bulletin No. 35, Hygienic Laboratory, P. H. and M. H. S. Report on the Origin and Prevalence of Typhoid Fever in the District of Columbia, 1906.

No detailed epidemiological investigations were made of the cases which were considered as certainly or almost certainly having contracted the infection out of the District. Therefore, most of the data in this report refer to the 523 cases comprised under *c*, *d*, and *e* of the above classification.

SEX.

Of the 523 cases, 304 occurred in males and 219 in females. Males compose about 48 per cent and females about 52 per cent of the population of the District of Columbia; 58.12 per cent of the cases were among males, showing, as was the case in the group studied by us in 1906, a relatively slightly higher typhoid fever morbidity rate among the males.

Two conditions, perhaps, contribute to the higher rate among the males; first, due to habits of occupation, etc., men are more exposed to infection, and, secondly, during the summer months, when the disease is most prevalent in Washington, a relatively larger number of women than men leave the city.

RACE.

Three hundred and eighty-seven, or 74 per cent, of the cases were among whites, and 136, or 26 per cent, among negroes. According to the police census for the spring of 1907, the population of the District of Columbia consists of 233,403 whites and 96,188 negroes, a total of 329,591. Thus negroes compose 29.18 per cent of the population, while the cases of typhoid fever among them composed 26 per cent of the total number investigated, showing a relatively somewhat lower morbidity rate among negroes than among whites. A discussion concerning the marked decrease in the morbidity and mortality rates in the negroes during the calendar year 1907 will be found on page 22, under " Prevalence."

AGES.

The following table gives the cases classified by age and sex, along with the population of the District of Columbia in 1900 (U. S. Census Report):

Ages, years.	Number of cases.			Population, 1900.			Ratio of cases to population.
	Male.	Female.	Total.	Male.	Female.	Total.	
0-4	11	11	22	11,683	11,467	23,150	1-1,052
5-9	48	33	81	11,708	12,023	23,731	1- 293
10-14	47	50	97	10,953	11,781	22,734	1- 234
15-19	41	33	74	71,362	13,452	24,814	1- 335

Ages, years.	Number of cases.			Population, 1900.			Ratio of cases to population.
	Male.	Female.	Total.	Male.	Female.	Total.	
20-24	51	28	79	13,774	17,736	31,510	1- 398
25-29	44	27	71	13,345	16,410	29,755	1- 419
30-34	27	13	40	11,911	12,857	24,768	1- 619
35-39	17	10	27	10,409	11,472	21,881	1- 810
40-44	3	4	7	8,679	9,255	17,934	1-2,562
45-49	8	8	16	6,876	7,750	14,626	1- 914
50-54	2	0	2	6,371	6,928	13,299	1-6,649
55-59	2	1	3	5,285	5,001	10,286	1-3,428
60-64	2	0	2	4,123	4,078	8,201	1-4,100
65-69	0	0	0	2,431	2,604	5,035	--------
70-74	1	1	2	1,611	1,829	3,440	1-1,720
75-79	0	0	0	796	1,049	1,845	--------
80-84	0	0	0	351	567	918	--------
85-89	0	0	0	132	206	338	--------
90-94	0	0	0	26	73	99	--------
95-99	0	0	0	10	29	39	--------
100 and over	0	0	0	6	14	20	--------
Age unknown	0	0	0	162	133	295	--------
Total	304	219	523	132,004	146,714	278,718	--------

The youngest person affected was 2 years and the oldest 73 years of age. As the cases occurred in 1907 and the census was held in 1900, the figures are not strictly comparable, but serve as a fair basis for an estimate. It will be noted that the largest number of cases proportionate to population occurred among persons from 10 to 14 years of age, and the next largest among persons from 5 to 9. Thus, our studies for the two years show that the opinion usually held that typhoid fever is most prevalent among persons in early adult life (20 to 30 years) is not true for the District of Columbia, the disease here being most prevalent among children. As typhoid fever in children is frequently mild and irregular, many cases are not recognized. Therefore it is probable that more cases than the table indicates actually occur in persons under the age of 15 years.

By decades, 103 of the cases were under 10 years, 171 between 10 and 20, 150 between 20 and 30, 67 between 30 and 40, 23 between 40 and 50, and 9 over 50.

The rather large proportion of cases among children points especially to two factors in the transmission of the infection of typhoid fever—contact and milk. Large numbers of children from many households are in free and intimate association in playing, so that the chances of spread of infection among them by direct or indirect contact must be greater than among adults.

Furthermore, mild and irregular attacks of typhoid fever are known to occur with greater frequency in children than in adults, so that the spread of infection by contact from unrecognized cases among children must be correspondingly more frequent.

Children drink more milk than adults, so that where milk is a frequent source of infection a relatively large number of cases among children may be expected.

In considering the number of cases among children in a community where typhoid fever has prevailed extensively for a number of years, we have to take into account that a proportionately larger number of adults have been rendered partially or completely immune by previous infection.

Contact between child and child and between child and adult is of a close and intimate nature, and such as to favor the spread of the infection of typhoid fever. Considering this fact, along with the known extensive prevalence of the disease among children, we have a possible explanation of the spread of the infection to many of the cases unaccounted for by epidemiological investigations, thus robbing the general problem of some of its mystery. Koch found that 49 of the 64 unrecognized cases of typhoid fever at Trier occurred among children. (Die Bekämpfung der Typhus, Berlin, 1903.) The large number of cases among children emphasizes the importance of keeping this factor in mind in carrying out practical measures to prevent the spread of the infection.

NATIONALITY.

In the following table is given the nationality of the cases along with the number of foreign born in the District of Columbia for 1900, according to the United States Census reports. For some of the nationalities the population no doubt has changed considerably since 1900, so that the figures in the table are not strictly comparable.

Nationality.	Number of cases.	Number of foreign-born in District of Columbia, census of 1900.
American	495	
Danish	1	88
English	3	2,299
German	5	5,857
Hungarian	1	48
Irish	5	6,220
Italian	11	930
Russian	1	807
Swedish	1	234
Total	523	

LENGTH OF RESIDENCE.

The length of residence in the District of Columbia of the persons affected was as follows:

Length of residence:	Cases.
Under six months	20
6 months to 1 year	15
1 to 2 years	28
2 to 3 years	16
3 to 4 years	19
4 to 5 years	17
5 to 10 years	62
10 to 15 years	26
15 to 20 years	16
20 to 30 years	18
30 to 40 years	7
40 to 50 years	4
50 to 60 years	1
Life	239
Not determined	35
	523

The ages of the 239 life residents of the District of Columbia affected were as follows:

Ages:	Cases.
0 to 4 years	17
5 to 9 years	55
10 to 14 years	67
15 to 19 years	38
20 to 24 years	23
25 to 29 years	22
30 to 34 years	8
35 to 39 years	7
40 to 44 years	1
45 to 49 years	1
	239

Nearly 50 per cent of the cases were among persons who had resided in the District of Columbia all their lives, so the disease can not be considered to have prevailed to any very unusual extent among newcomers. However, it should be noted that the number of cases, 115, among persons who had resided in the District less than five years was nearly twice as large as the number, 62, among persons who had resided in the District for five to ten years, and over four times as large as the number, 26, among those who had resided in the District from ten to fifteen years.

In considering these figures we should take into account that the transient element of Washington's population is rather large, and that among persons who have lived in the District less than ten years there is, of course, a large proportion of children.

PREVALENCE.

The following table shows the cases according to dates of definite onset:

Day of month.	May.	June.	July.	August.	September.	October.
1	2	0	0	11	5	5
2	1	0	1	5	5	8
3	2	0	1	6	3	3
4	1	1	2	6	5	3
5	2	2	3	7	3	2
6	0	0	3	2	4	2
7	0	1	3	6	4	3
8	0	1	3	9	1	2
9	1	2	5	4	2	2
10	3	2	6	7	11	6
11	0	1	2	4	1	1
12	1	2	3	8	2	2
13	0	3	2	11	3	4
14	2	0	2	6	2	3
15	0	0	3	7	3	6
16	1	2	2	3	2	3
17	1	2	2	5	4	3
18	0	1	3	10	4	0
19	1	0	4	2	5	0
20	1	3	5	5	8	7
21	0	1	3	5	4	2
22	0	0	1	5	3	1
23	0	2	7	1	1	0
24	0	2	1	3	1	1
25	1	3	7	6	6	0
26	1	3	3	2	1	0
27	2	3	7	7	4	0
28	0	2	7	5	6	0
29	3	2	7	5	2	0
30	1	1	1	2	3	0
31	0	----------	3	1	------------	0

As in our previous report, the cases are tabulated and charted according to date of onset of definite symptoms, it having been found again that in the majority of instances accurate statements as to date of onset of the first slight symptoms could not be obtained.

The following table shows the progress of the disease as indicated by dates of onset of the cases in periods of a half month. The number of cases for corresponding periods in 1906 are placed in a parallel column:

Periods.	Cases.	
	1907.	1906.
February 1 to 15, incomplete	1	--------
April 1 to 15, incomplete	1	--------
April 16 to 30, incomplete	7	--------
May 1 to 15, complete	15	--------

Periods.	Cases.	
	1907.	1906.
May 16 to 31, complete	12	-------
June 1 to 15, complete	15	32
June 16 to 30, complete	27	32
July 1 to 15, complete	39	101
July 16 to 31, complete	63	131
August 1 to 15, complete	99	139
August 16 to 31, complete	67	70
September 1 to 15, complete	54	59
September 16 to 30, complete	54	41
October 1 to 15, incomplete	[a] 52	98
October 16 to 31, incomplete	[a] 17	22

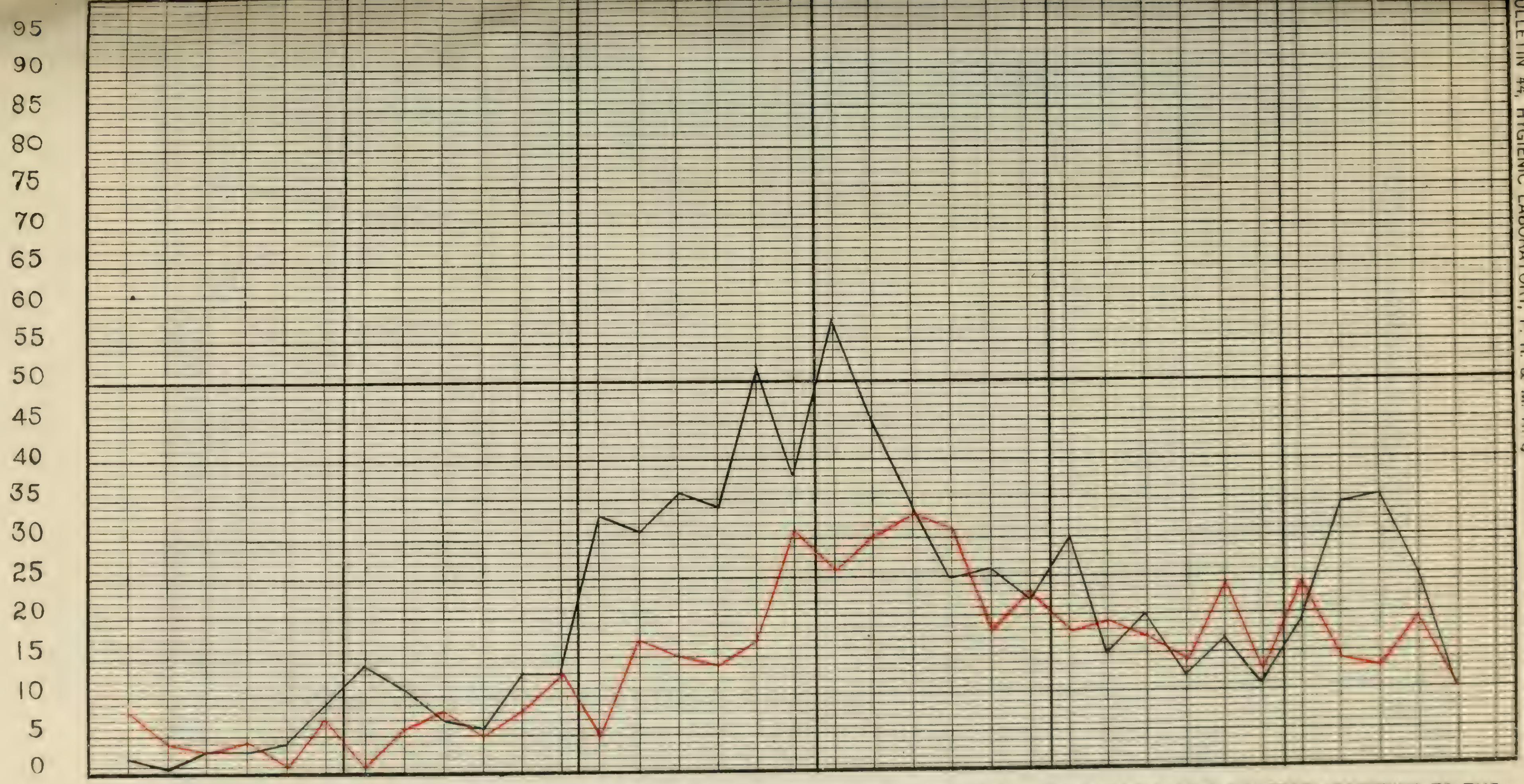
[a] Completed figures for October, including cases reported subsequent to November 1, are: October 1 to 15, 64; October 16 to 31, 42.

The rate began to increase in the latter half of June and gradually rose through July and the early part of August, reaching its maximum about the middle of August and then rather gradually declined through the latter half of August and through September. A comparison of the rates for the summers of 1906 and 1907 shows that in 1907 the increase in the rate was somewhat later, decidedly less, and with a rise and fall more gradual than that of 1906. (See Charts Nos. 1 and 2.) The total number of cases occurring in June, July, and August, 1907, was 310, while the total for the corresponding period of 1906 was 505, a difference of 195 cases. During the winter, spring, and fall seasons the rate of prevalence for the two years was about the same. (See Table, p. 23.)

The results of our investigations suggest that the lower rate for the summer of 1907 for the most part was not due to a decrease in the operation of two known factors in the spread of typhoid fever infection, viz, milk and contact. Of the 505 cases in the summer months of 1906 there were 48, or 9.5 per cent, that occurred in the course of two pronounced milk outbreaks, and were attributed to infected milk. Of the 310 cases in the corresponding period of 1907, 28, or 9.03 per cent, occurred in the course of a pronounced milk outbreak and were attributed to milk infection.

Of the 747 cases investigated by us and considered as having contracted the infection in the District of Columbia in 1906, 7.3 per cent were fairly definitely attributable to infection by direct or indirect contact; while of the 523 cases in 1907, 19 per cent were fairly definitely attributable to infection by direct or indirect contact. Thus, s far as the results of our investigation indicate, milk as a factor in pronounced outbreaks operated relatively to about the same extent during the two summers and contact operated as a factor relatively to certainly as great an extent in the 1907 period as it did in the 190

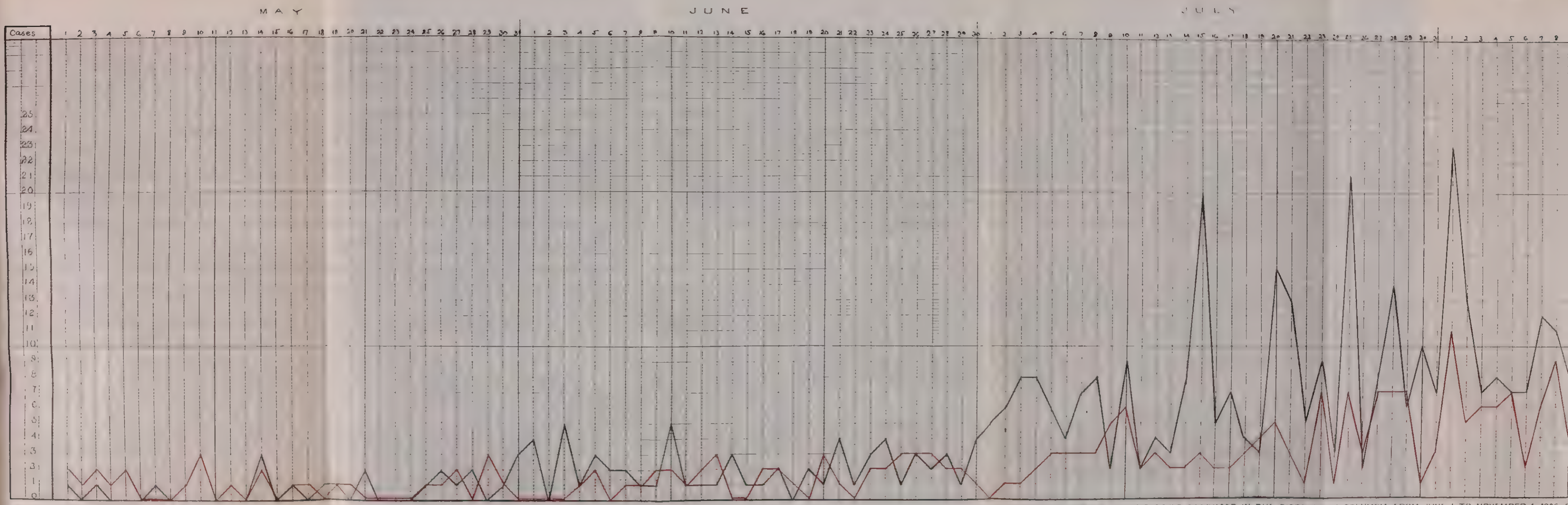
CHART No. 1



CASES OF TYPHOID FEVER REPORTED IN THE DISTRICT OF COLUMBIA FROM JUNE 1 TO NOVEMBER 1, 1906, CHARTED ACCORDING TO THE DATE OF ONSET OF ILLNESS, IN FIVE-DAY PERIODS.

CASES OF TYPHOID FEVER REPORTED IN THE DISTRICT OF COLUMBIA FROM MAY 1 TO NOVEMBER 1, 1907, CHARTED ACCORDING TO THE DATE OF ONSET OF ILLNESS, IN FIVE-DAY PERIODS.

THE NORRIS PETERS CO., WASHINGTON, D. C.



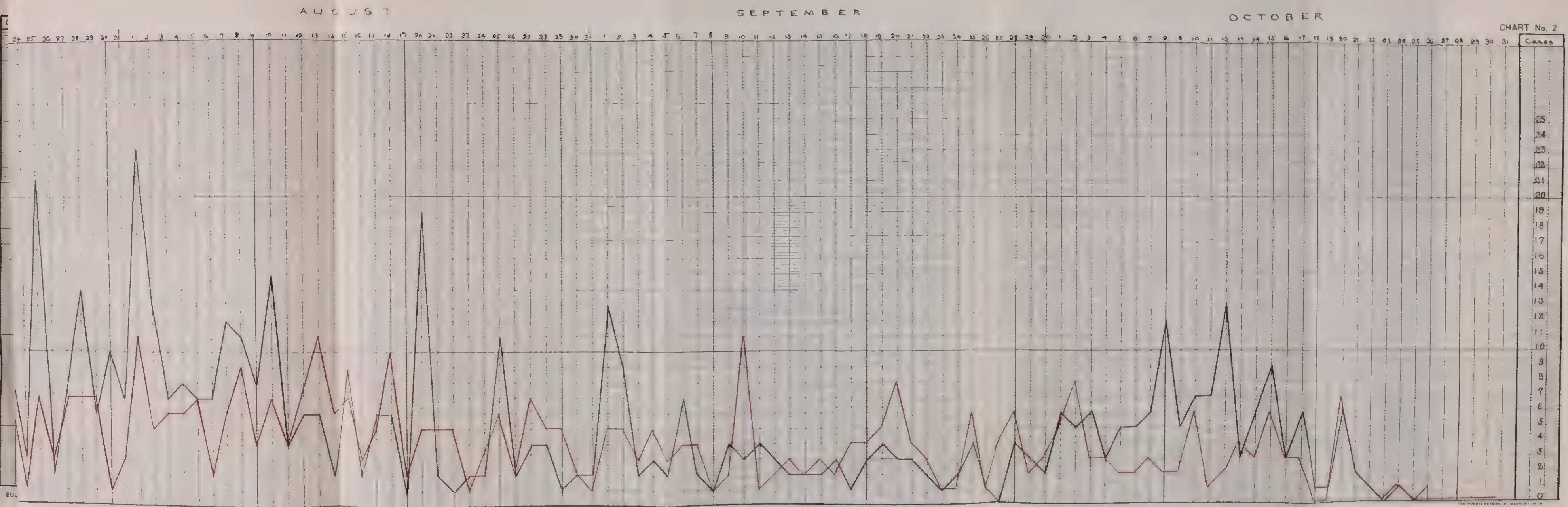
BULLETIN 44, HYGIENIC LABORATORY, P. H. & M. H. S.

CASES OF TYPHOID FEVER REPORTED IN THE DISTRICT OF COLUMBIA FROM JUNE 1 TO NOVEMBER 1, 1906, C

CASES OF TYPHOID FEVER REPORTED IN THE DISTRICT OF COLUMBIA FROM MAY 1 TO NOVEMBER 1, 1907, C

20³

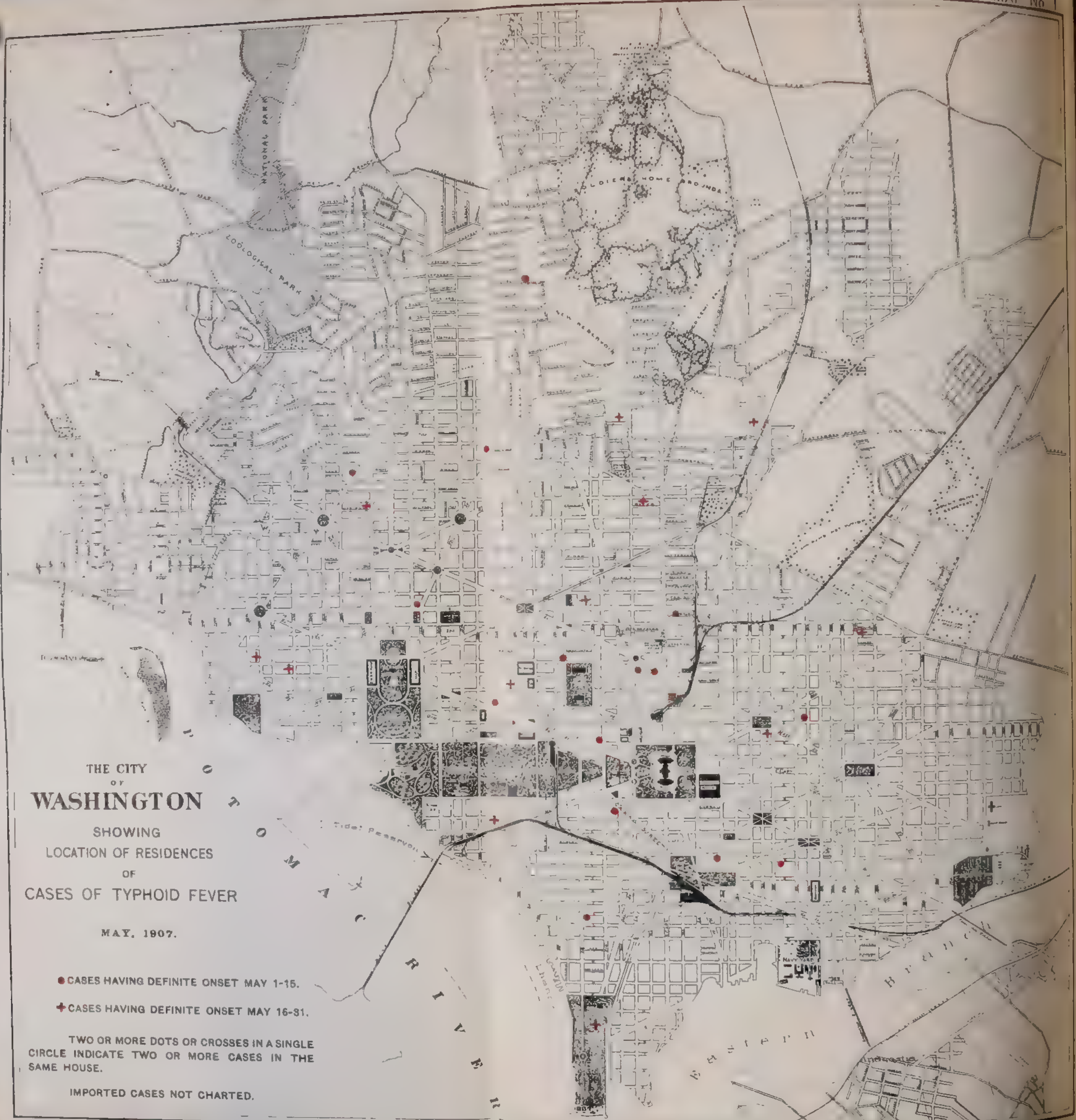
CHART No. 2



OF COLUMBIA FROM JUNE 1 TO NOVEMBER 1, 1906, CHARTED ACCORDIN TO DATE OF ONSET OF DISEASE

OF COLUMBIA FROM MAY 1 TO NOVEMBER 1, 1907, CHARTED ACCORDING TO DATE OF ONSET OF DISEASE

MAP No. 1



THE CITY
OF
WASHINGTON
SHOWING
LOCATION OF RESIDENCES
OF
CASES OF TYPHOID FEVER

MAY, 1907.

● CASES HAVING DEFINITE ONSET MAY 1-15.

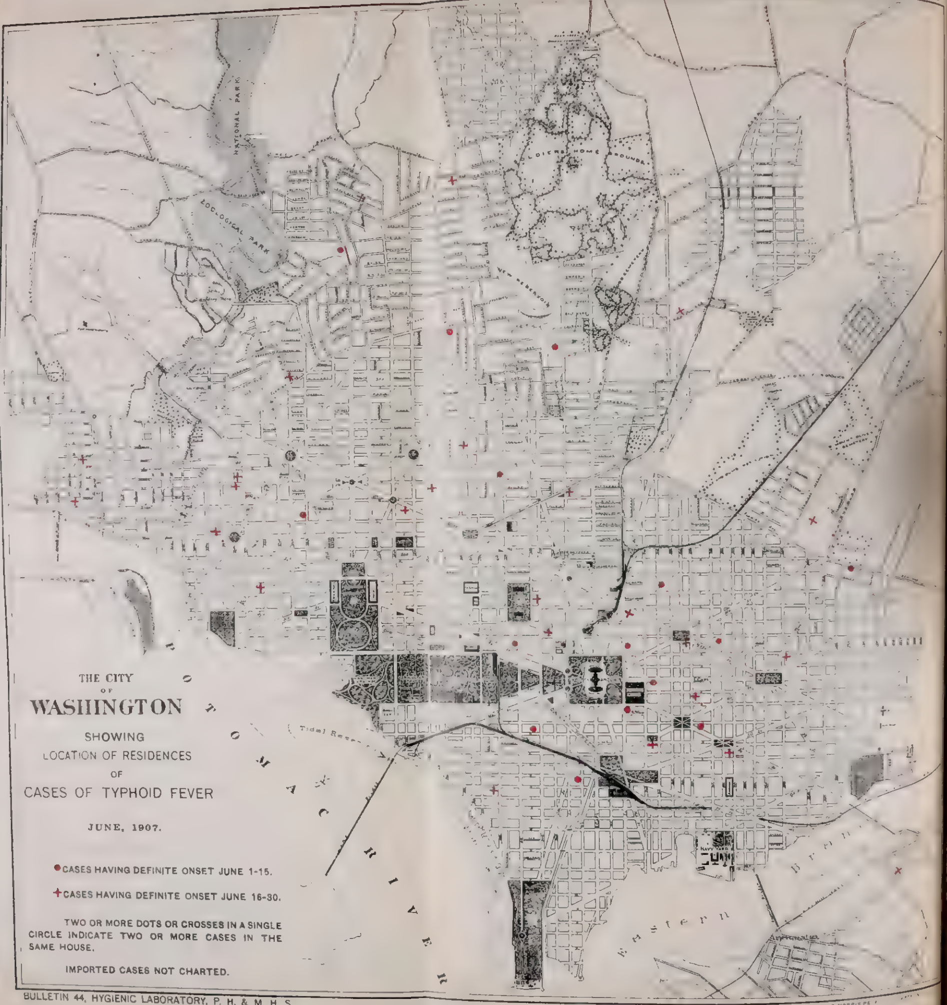
✚ CASES HAVING DEFINITE ONSET MAY 16-31.

TWO OR MORE DOTS OR CROSSES IN A SINGLE CIRCLE INDICATE TWO OR MORE CASES IN THE SAME HOUSE.

IMPORTED CASES NOT CHARTED.

BULLETIN 44, HYGIENIC LABORATORY, P H & M H S

THE NORRIS PETERS CO., WASHINGTON, D. C.



THE CITY
OF
WASHINGTON

SHOWING
LOCATION OF RESIDENCES
OF
CASES OF TYPHOID FEVER

JUNE, 1907.

● CASES HAVING DEFINITE ONSET JUNE 1-15.

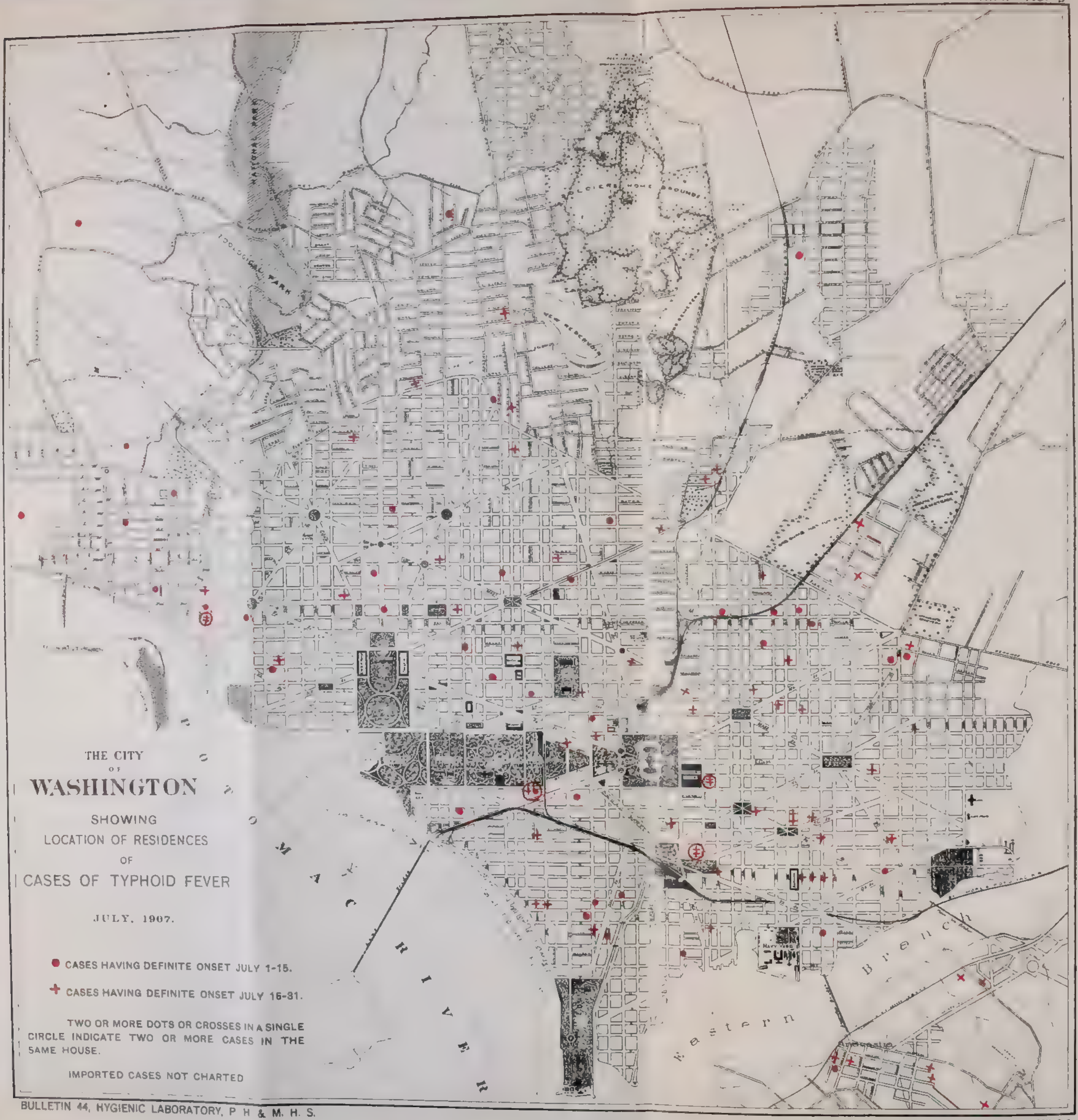
+ CASES HAVING DEFINITE ONSET JUNE 16-30.

TWO OR MORE DOTS OR CROSSES IN A SINGLE CIRCLE INDICATE TWO OR MORE CASES IN THE SAME HOUSE.

IMPORTED CASES NOT CHARTED.

THE NORRIS PE

MAP No. 3



THE CITY
OF
WASHINGTON
SHOWING
LOCATION OF RESIDENCES
OF
CASES OF TYPHOID FEVER

JULY, 1907.

● CASES HAVING DEFINITE ONSET JULY 1-15.

+ CASES HAVING DEFINITE ONSET JULY 16-31.

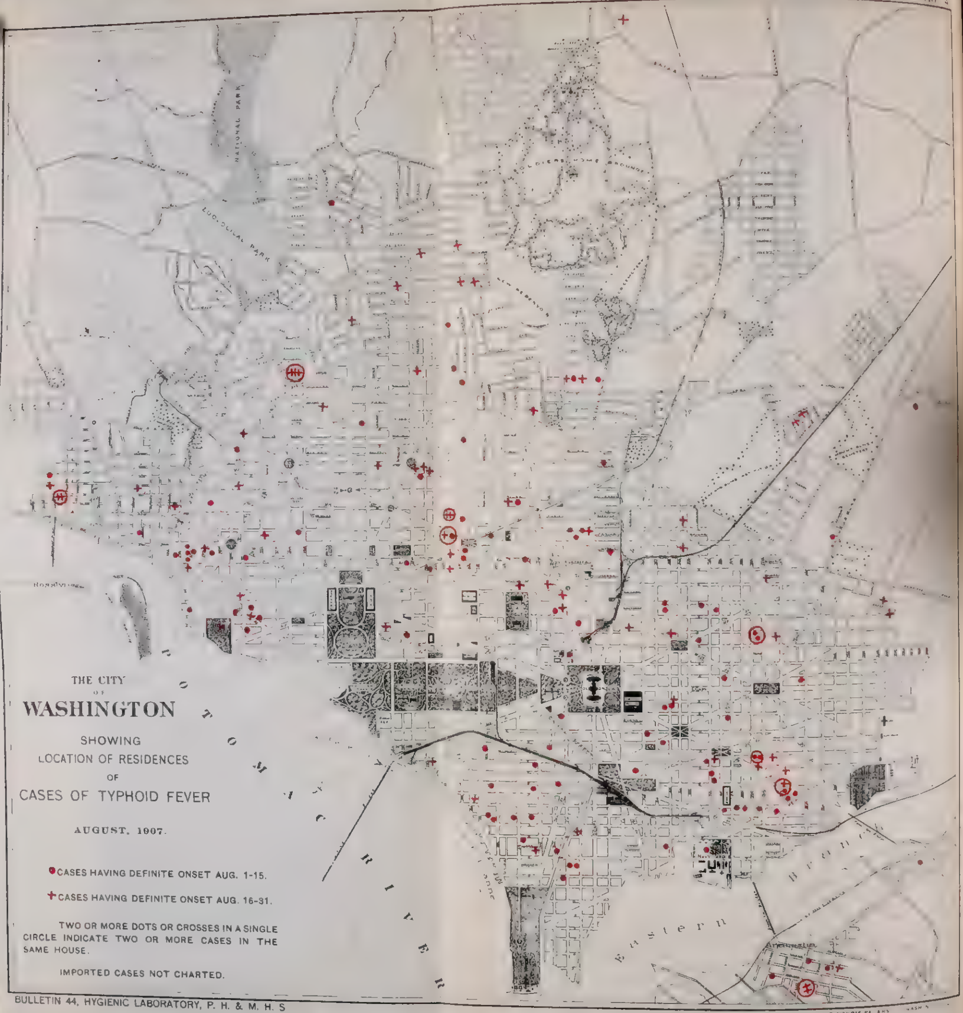
TWO OR MORE DOTS OR CROSSES IN A SINGLE CIRCLE INDICATE TWO OR MORE CASES IN THE SAME HOUSE.

IMPORTED CASES NOT CHARTED

BULLETIN 44, HYGIENIC LABORATORY, P H & M. H. S.

THE NORRIS PETERS C., WASHINGTON D C

MAP No. 4



THE CITY
OF
WASHINGTON
SHOWING
LOCATION OF RESIDENCES
OF
CASES OF TYPHOID FEVER

AUGUST, 1907.

● CASES HAVING DEFINITE ONSET AUG. 1-15.

+ CASES HAVING DEFINITE ONSET AUG. 16-31.

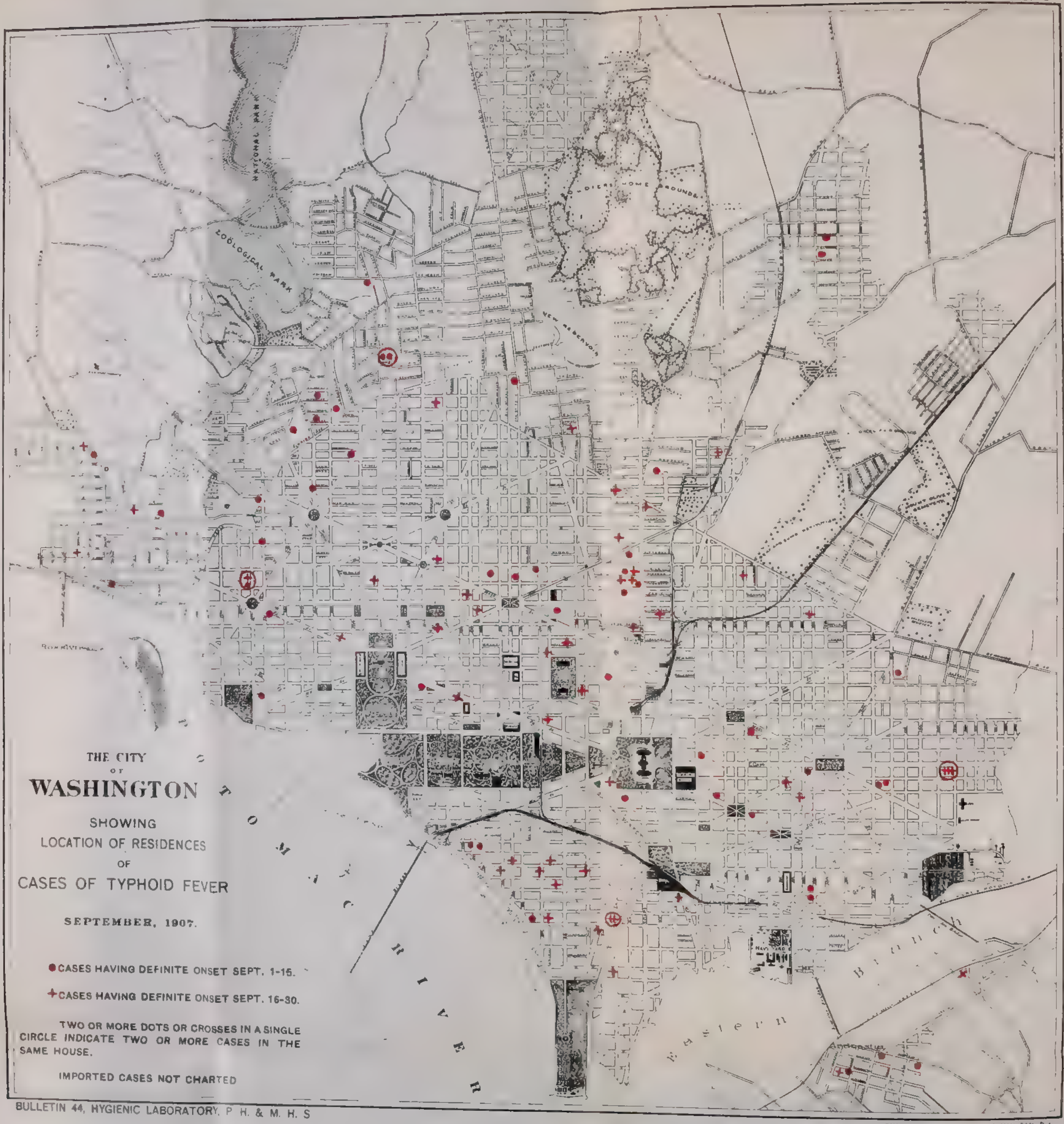
TWO OR MORE DOTS OR CROSSES IN A SINGLE CIRCLE INDICATE TWO OR MORE CASES IN THE SAME HOUSE.

IMPORTED CASES NOT CHARTED.

BULLETIN 44, HYGIENIC LABORATORY, P. H. & M. H. S

THE NORRIS PETERS CO. WASH. D.C.

MAP No. 5



THE CITY
OF
WASHINGTON
SHOWING
LOCATION OF RESIDENCES
OF
CASES OF TYPHOID FEVER

SEPTEMBER, 1907.

● CASES HAVING DEFINITE ONSET SEPT. 1-15.

✚ CASES HAVING DEFINITE ONSET SEPT. 16-30.

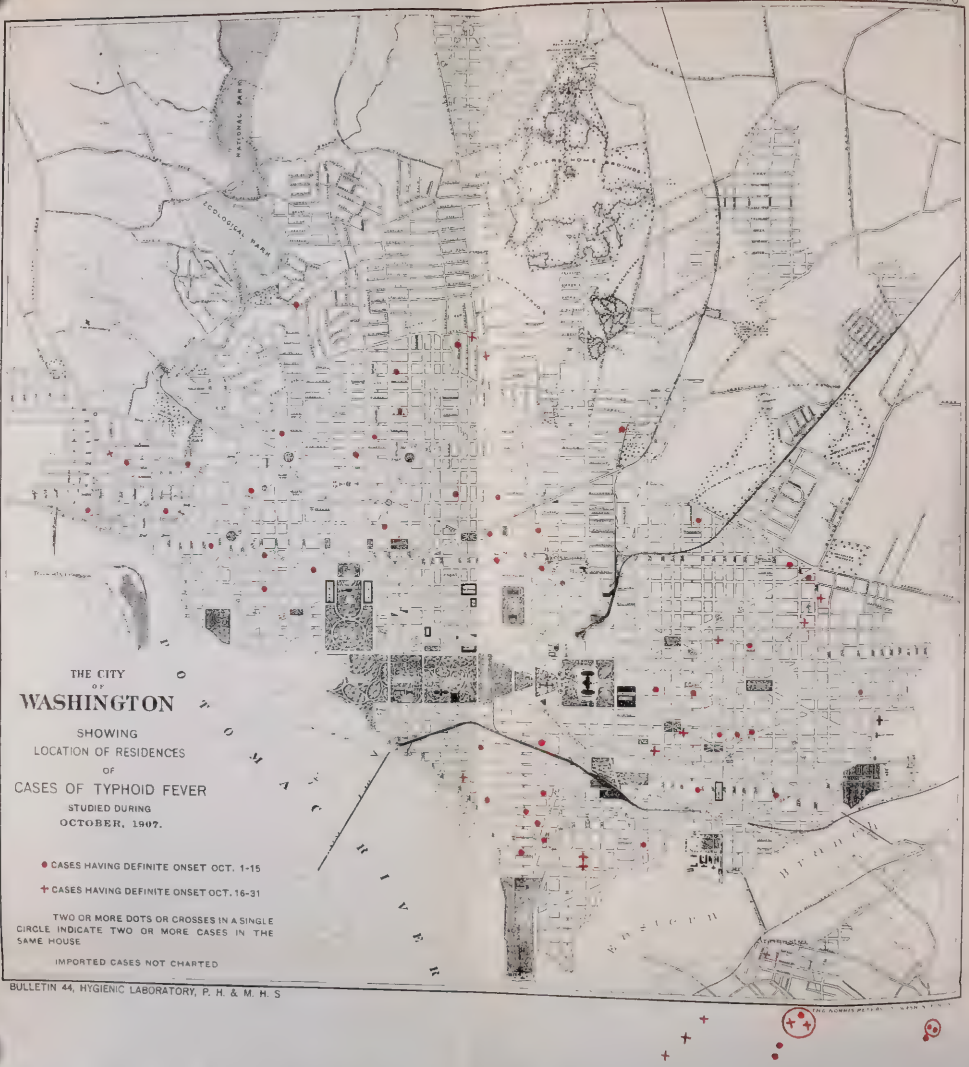
TWO OR MORE DOTS OR CROSSES IN A SINGLE CIRCLE INDICATE TWO OR MORE CASES IN THE SAME HOUSE.

IMPORTED CASES NOT CHARTED

BULLETIN 44, HYGIENIC LABORATORY, P. H. & M. H. S

THE NORRIS PETERS WASHINGTON D.C.

MAP No. 6



THE CITY
OF
WASHINGTON

SHOWING
LOCATION OF RESIDENCES
OF
CASES OF TYPHOID FEVER
STUDIED DURING
OCTOBER, 1907.

● CASES HAVING DEFINITE ONSET OCT. 1-15

+ CASES HAVING DEFINITE ONSET OCT. 16-31

TWO OR MORE DOTS OR CROSSES IN A SINGLE CIRCLE INDICATE TWO OR MORE CASES IN THE SAME HOUSE

IMPORTED CASES NOT CHARTED

BULLETIN 44, HYGIENIC LABORATORY, P. H. & M. H. S

THE NORRIS PETERS CO., WASH. D.C.

period. Therefore, it appears that the difference in rate for the two summers may have been due to a difference in extent of operation of some factor or factors other than milk and contact.

That the difference in prevalence of typhoid fever in Washington for the two summers was probably not due to a difference in temperature or humidity is indicated by reports from Baltimore that typhoid fever was fully as prevalent in that city in the summer of 1907 as in that of 1906. The rise in both cities was belated in the summer of 1907.

So far as can be judged by reports received from various cities there has been no material difference in the prevalence of typhoid fever throughout the country during the two years. In some places there has been more, in others less, and in some about the same during the two years.

In the 1907 period there was relatively a considerably smaller number of cases among the wealthy class of people than in the 1906 period. Maps 1, 2, 3, 4, 5, 6, and 7 show the distribution of cases by place of residence when the disease was contracted. A comparison of these maps with those showing the distribution of the cases studied in 1906 (see Bulletin No. 35) shows a somewhat less uniform distribution of cases during the half monthly periods of 1907. In 1907 there was a quite general distribution of cases throughout the city, but in proportion to population there was relatively a larger number of cases in the more unhygienic sections of the city.

Therefore, it appears from our observations this year that typhoid fever may be quite uniformly and generally scattered throughout a large city having water of good sanitary quality, so far as can be judged by our present bacteriological standards. It has been assumed that a more or less widespread and uniform distribution of an infectious disease like typhoid fever points to a common factor, such as infected water. Whether this general distribution was due primarily to contaminated water in previous months or years, or to other causes can only be determined by future studies.

The Government Hospital for the Insane, with a population of about 3,000 but with artesian well water, pasteurized milk and generally good sanitary conditions, again this season remained free of infection. One case occurred among the employees of the institution, but this case was a man who made frequent visits to, and took meals at his home in, Washington.

The total number of cases of typhoid fever in the District of Columbia reported to the health office during the calendar year 1907 was 945 as against 1,126 for the calendar year 1906. Thus, the typhoid fever morbidity rate in the District of Columbia for the calendar year 1907 was 2.87 per 1,000 of population, or one person in every 348; while in 1906 the morbidity rate was 3.45 per 1,000, or one in every 289.

The number of deaths from typhoid fever in the District of Columbia during 1907 was 114, being a death rate of 34.5 per 100,000 of population, as against 49.3 for 1906. The percentage of deaths to cases (mortality rate) for the two years was as follows:

Year.	White.	Colored.	Total.
1907	10.4	17.6	12.0
1906	10.3	24.2	14.4

Thus the mortality rate was higher in 1906 than in 1907, and as the rate for the whites was slightly lower in 1906 than in 1907, the higher rate in 1906 was due to the higher rate among the colored. As the mortality rate for the whites was about the same for the two years, it does not seem probable that the lower rate for the colored in 1907 was due to the occurrence of a milder type of infection in Washington during that year. From what we know of the facts the difference was not due to improvement in the treatment of cases in 1907. Therefore it seems that the different rates among the colored for the two years may have been due to difference in percentage of error in diagnosis and report of cases.

The mortality rates for the other three years of which there is record were as follows:

Year.	White.	Colored.	Total.
1903	10.69	19.35	12.98
1904	10.89	22.42	14.01
1905	11.66	16.33	12.94

The rate among the whites during the five years is seen to be fairly constant, while among the colored it varies considerably from year to year. Probably the lower figures more nearly approach the actual mortality rate among the negroes, and in those years in which the figures are high it is likely that the proportion of cases reported to the number which actually occurred was relatively low. This is based on the assumption that typhoid fever may not show marked variations in virulence from year to year among the colored people in an endemic area.

In the following table is given the number of cases and deaths from typhoid fever in the District of Columbia reported by months to the health office during the calendar years 1906 and 1907:

Month.	Cases.						Deaths.					
	White.		Colored.		Total.		White.		Colored.		Total.	
	1906.	1907.	1906.	1907.	1906.	1907.	1906.	1907.	1906.	1907.	1906.	1907.
January	24	46	3	7	27	53	5	6	1	1	6	7
February	15	28	6	4	21	32	2	3	2	3	4	6
March	13	23	5	2	18	25	2	2	3	2	5	4
April	29	20	6	8	35	28	2	3	2	3	4	6
May	31	31	13	6	44	37	6	5	4	2	10	7
June	32	30	26	4	58	34	4	1	5	1	9	2
July	123	61	58	21	181	82	7	6	14	4	21	10
August	200	145	93	46	293	191	15	13	17	5	32	18
September	105	146	44	41	150	187	10	13	10	4	20	17
October	129	102	43	47	171	149	16	12	12	7	28	19
November	57	68	25	19	82	87	11	8	8	3	19	11
December	38	35	8	5	46	40	2	5	2	2	4	7
Total	796	735	330	210	1,126	945	82	77	80	37	162	114

As this table gives the cases by date of report of cases to the health office, which date is usually two or three weeks (and often more) subsequent to the onset of illness, and as it includes imported cases, it shows but roughly the actual progress of the disease in the District of Columbia. Table on page 19 is much more accurate in this respect. The above table shows that the number of cases and deaths from typhoid fever reported among the negroes in 1907 was much smaller than in 1906, a large part of the difference in total cases and deaths for the two years being due to the lower rate for the negroes in 1907. It is interesting to note that the disproportionately small number of colored to white cases in 1907 was among cases reported in the winter, spring, early summer, and late fall months—that is, out of the season in which typhoid fever has in the District of Columbia its greatest prevalence (by report of cases), namely, in July, August, September, and October. Thus, in 1907 there were reported in the eight months of January, February, March, April, May, June, November, and December 281 white cases and 58 colored cases, or a ratio of 1 colored to 4.8 white cases, while in the four months of July, August, September, and October there were reported 454 white and 155 colored cases, a ratio of 1 colored to 2.9 white cases.

In 1906 there was no such striking difference in the proportion of colored to white cases for the two corresponding portions of the year. Thus, in the eight months of January, February, March, April, May, June, November, and December, 1906, there were reported 239 white cases and 92 colored cases, a ratio of 1 colored to 2.4 white cases, while in the four months of July, August, September, and October, 1906, there were reported 537 white and 238 colored cases, a ratio of 1 colored to 2.2 white cases. In 1905 the ratio of colored to white cases for the first of the above periods was 1 to 2.9, and for the second period (July, August, September, and October), 1 to 2.5.

Considering these facts, it is evident that during the cool weather, or what may be called the *off season* for typhoid fever in Washington—that is, in January, February, March, April, May, October, November, and December, of 1907, the factors which operate to cause typhoid fever in the District of Columbia fell either disproportionately heavily upon the whites or disproportionately lightly upon the colored. In 1907 we investigated only the cases reported from May 1 to November 1, and as the marked disproportion in the cases reported among whites and colored was principally in the months not covered by our studies, we have no data of our own on which to base an explanation of this most remarkable occurrence.

For a discussion of seasonal prevalence in relation to water borne typhoid see page 45.

SANITARY CONDITION OF RESIDENCES.

In the following table is given the general sanitary condition of the residences at which the patients had lived when the infection probably was contracted, the condition of residences of cases studied in 1906 being placed in the parallel column:

Condition of residence.	Number of cases.	
	1907.	1906.
Good [a]	98	253
Fairly good [a]	228	24
Rather bad [a]	140	15
Bad [a]	57	9
Not determined [a]	0	
	523	74

[a] The definitions of these terms correspond to those used in our previous report (Bulletin 35).

The percentage of cases among persons at residences of these fou classes for the two periods was as follows:

Condition.	Percentage of cases.	
	1907.	1906.
Good	18.7	33.
Fairly good	43.5	32.
Rather bad	26.7	21.
Bad	10.9	12.

The proportion of cases among persons living at residences of which all the sanitary conditions were good was considerably smaller during the 1907 period than during the 1906 period; but again this year a majority (about 62 per cent) of the cases occurred among persons living at residences of good or fairly good sanitary condition.

DISPOSAL OF SEWAGE.

Of the 523 cases, 462 were among persons living at residences connected with the city sewerage system and at which there were water-closets, 56 at residences not connected with the city sewerage system and at which there were box privies, 1 at a residence from which the water-closet discharged into a subsoil cesspool, 1 at a residence having neither water-closet nor privy, and 3 at institutions not connected with the city sewerage system but having water-closets which discharged into local sewerage systems.

Of the 462 cases occurring at residences connected with the city sewerage system, 212 were at residences having water-closets in the house only, 163 at residences having water-closets in the yard only, and 87 at residences having water-closets in both house and yard. As in the 1906 period, there was no unusual prevalence of the disease among persons living at residences not connected with the city sewerage system and at which privies or other local means of disposing of sewage were in use. Thus, in the 1907 period 88.3 per cent and in the 1906 period 90 per cent of the cases were at residences connected with the city sewerage system and supplied with water-closets.

Of the 56 cases occurring at houses at which privies were in use, 15 were secondary cases and attributed to personal infection from cases in the same house or in a house nearby.

The danger of the spread of the infection by flies, etc., necessarily is greater where the excreta of the typhoid fever patients are deposited in privies, and it is therefore important to abolish privies as speedily as the extension of the city's sewerage system makes possible.

WATER.

Of the 523 cases, 492, or about 94 per cent, gave a history of having used unboiled Potomac water supplied through the regular city system as the sole, principal, or occasional source of water for drinking during the thirty days prior to the onset of illness. Of the cases investigated during the 1906 period, 96.54 per cent gave a history of having used the unboiled Potomac water.

The following table gives the source of water used for drinking during the thirty days prior to onset of illness by the 523 cases investigated:

Raw tap:	
Solely	253
Principally	187
Occasionally	52
Occasionally (?)	4
Boiled tap:	
Solely	3
Principally	13
Occasionally	2
Filtered tap:	
Solely	1
Principally	2
Occasionally	0
Public wells or springs:	
Solely	7
Principally	5
Occasionally	36
Bottled:	
Solely	0
Principally	1
Occasionally	8
Private wells or springs in the District of Columbia:	
Solely	12
Principally	29
Occasionally	15
Various sources out of District of Columbia:	
Principally	8
Occasionally	157

It is interesting to compare some of the figures in this table with those in a similar table for the cases studied by us in 1906. Thus, there is little difference in the proportion of cases among persons who used the raw tap water. In the 1907 period 4.3 per cent and in the 1906 period 1.3 per cent of the cases were among persons who had used water other than the raw tap as the sole source for drinking.

In the 1907 period, 165, or 31.5 per cent, of the 523 cases investigated were in persons who, during the thirty days prior to the onset of the disease, had used water principally or occasionally for drinking from some source outside of the District of Columbia as against 69, or only 9.2 per cent, of the cases for the 1906 period.

SHALLOW WELL WATERS.

The following list of public shallow wells closed during 1907 is given as furnished us by the health officer:

NORTHWEST SECTION.

Location.	Date closed.
Brightwood avenue, near District line	July 20, 1907
Eighth street and Barry place	July 17, 1907
Clark street and Elliott place	July 22, 1907
Massachusetts avenue, between Sixth and Seventh streets	July 17, 1907
New York avenue, between Fourth and Fifth streets	July 15, 1907
New Jersey avenue, between M and N streets	Do.
North Capitol street and Randolph place	Nov. 11, 1907
Ninth and H streets	July 15, 1907
Newton street, east of Brightwood avenue	July 20, 1907
Sixth, between F and G streets	July 15, 1907
Sixth, between Fairmont and Girard streets	July 17, 1907
Sixteenth and Corcoran streets	July 16, 1907
Seventeenth and K streets	Do.
T, between Seventeenth and Eighteenth streets	Do.
Third street and Indiana avenue	July 15, 1907
Third and L streets	Do.
Tenth and K streets	Do.
Tenth and N streets	July 17, 1907
Twelfth street and Florida avenue	Nov. 11, 1907
Twenty-eighth and O streets	July 18, 1907
Wisconsin avenue and P street	Do.
Wisconsin avenue and Q street	Do.
Wisconsin avenue and Thirty-third street	Do.
Thirty-fourth and Volta streets	Do.
Thirty-fourth street and Wisconsin avenue	July 19, 1907
Thirty-fifth and Reservoir streets	Do.
Thirty-second, north of Q street	July 18, 1907

NORTHEAST SECTION.

E, between Eighth and Ninth streets	July 20, 1907
Eighth and A streets	July 19, 1907
Eleventh and F streets	Do.
Fourth and E streets	Do.
Keating, between Lincoln road and First street	July 18, 1907
North Capitol, between B and C streets	July 17, 1907
South Dakota avenue and Vista street	July 24, 1907
Second and G streets	July 18, 1907
Sixth and C streets	Do.
Third and D streets	July 17, 1907

SOUTHEAST SECTION.

Eighth and D streets	July 20, 1907
Eleventh street and South Carolina avenue	July 18, 1907
Fillmore and Jackson streets	July 19, 1907
Fourth and C streets	July 16, 1907
Fourth street and South Carolina avenue	July 18, 1907
Fifth and G streets	Do.

SOUTHEAST SECTION—Continued.

Location.	Date closed.
I, between Eleventh and Twelfth streets	July 23, 1907
Jefferson, between Monroe and Fillmore streets	July 19, 1907
L, between Thirteenth and Fourteenth streets	July 22, 1907
Ninth and E streets	Do.
O, between Half and First streets	July 24, 1907
Seventh and B streets	July 23, 1907
Seventh street, between North Carolina avenue and C street	July 16, 1907
Seventeenth and Harrison streets	July 19, 1907
Third and C streets	July 16, 1907
Third street and Pennsylvania avenue	July 17, 1907
Thirteenth, between D and E streets	July 23, 1907

It will be noticed that the closing of these wells occurred too late to account for the improvement in the situation this season.

The following is a list of shallow wells in the District of Columbia still in operation, and, excepting the one on Quincy street, between Twelfth and Thirteenth streets NE., all are out of immediate touch with the public water-main system. There are various shallow wells at public schools of which there is no record at the health office, and they are not included in this list:

Northwest.—Massachusetts avenue, extended, near Wisconsin avenue; Tenleytown road, Tenleytown.

Northeast.—Benning cross roads; Quincy street between Twelfth and Thirteenth streets.

Southeast.—Elvan avenue, west of Stanton avenue; Hamilton road, near Jewish cemetery; Stanton avenue and Pomeroy avenue; Twentieth and Joliet streets, Garfield; T street hill, Hillsdale.

BACTERIOLOGIC EXAMINATION OF THE POTOMAC WATER SUPPLY.

This season the bacteriologic examination of water was confined to that supplied from the Potomac River, this now being almost the sole supply of water for the city of Washington.

The examinations were made in the Division of Pathology and Bacteriology of the Hygienic Laboratory by Passed Assistant Surgeons George W. McCoy and A. M. Stimson and Assistant Surgeon W. W. Miller. As in the previous year, the technique recommended by the committee on standard methods of water analysis of the American Public Health Association on January 9, 1905, was followed. From May 15 to November 30 two samples of water were obtained and examined daily, except Sundays. One sample was taken from the inlet at Dalecarlia Reservoir, this representing the Potomac River water as it is introduced into the first storage reservoir, and one sample from the outlet of the filtered-water reservoir, this representing the mixed effluents from all the filter beds and being the water just before it passes from the reservoir to the conduits for distribution to the city.

In the following tables are given the results of the bacteriologic xaminations of samples of the raw river water and of the filtered ater:

THE RAW RIVER WATER.

able showing the number of bacteria and presence of colon bacilli found in samples taken from the inlet of Dalecarlia Reservoir.

aily examinations made by the (*a*) Laboratory of the Filtration Plant; (*b*) Bureau of Plant Indu-try of Agricultural Department; (*c*) Hygienic Laboratory, Public Health and Marine-Hospital Service.]

MAY.

Date.	Number of bacteria per cubic centimeter.			*B. coli* in—			Fermentation in sugar broth (*c*).		
	(*a*) Filtration plant.	(*b*) Agricultural Department.	(*c*) Hygienic Laboratory.	0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1907.									
ay 15	900	410	1,700	....	+(*c*) (*a*)....	+(*a*) (*b*) (*c*)	—	+	+
16	800	240	1,800	....	+(*c*).......	+(*a*) (*b*) (*c*)	—	+	+
17	460	515	1,080	....	+(*c*).......	+(*a*) (*b*) (*c*)	—	+	+
18	210	110	520	....	+(*a*) (*b*) (*c*)	+(*a*) (*b*) (*c*)	—	+	+
19	....	....	....	....	....	....	....	....	....
20	850	122	1,000	....	+(*a*) (*c*)...	+ (*a*) (*c*) ..	—	+	+
21	1,300	372	1,720	....	+(*c*).......	+(*a*) (*b*) (*c*)	—	+	+
22	850	188	1,850	+(*a*) (*c*).	+(*a*) (*c*)...	+(*a*) (*b*) (*c*)	+	+	+
23	300	240	460	....	+(*a*)	+(*a*) (*b*) (*c*)	—	—	+
24	280	340	580	....	+(*a*) (*b*)...	+(*a*) (*b*) (*c*)	—	—	+
25	550	175	280	....	....	—(*a*) (*b*) (*c*)	—	—	+
26	....	....	....	....	....	....	....	....	....
27	550	268	290	....	+(*c*).......	+ (*b*) (*c*) ..	—	+	+
28	700	184	470	....	+(*a*) (*c*)...	+ (*a*) (*b*) ..	—	+	—
29	750	84	580	....	+(*a*) (*c*)...	+ (*a*) (*c*) ..	—	+	+
30	....	....	....	....	....	....	....	....	....
31	650	195	460	....	+(*a*) (*c*)...	+(*a*) (*b*) (*c*)	—	+	+
Average....	653+	245+	913+						

Table showing the number of bacteria and presence of colon bacilli found in samples taken from the inlet of Dalecarlia Reservoir—Continued.

JUNE.

Date.	Number of bacteria per cubic centimeter.			*B. coli* in—			Fermentation in sugar broth (*c*).		
	(*a*) Filtration plant.	(*b*) Agricultural Department.	(*c*) Hygienic Laboratory.	0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1907.									
June 1	420	61	....	....	+(*a*) (*b*) ...	+ (*a*) (*b*)...	+	+	+
2									
3	9,000	327	12,600	+ (*c*)....	+(*a*)(*b*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
4	12,000	317	14,300	+ (*c*)....	+(*a*)(*c*)...	+(*b*)(*a*)(*c*)	+	+	+
5	8,000	1,145	11,200	+(*a*)(*c*)..	+(*b*)(*a*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
6	3,100	966	7,350	+(*a*)(*c*)..	+(*b*)(*a*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
7	3,600	666	5,800	+(*c*).....	+(*a*)(*b*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
8	1,700	535	390	....	+(*b*)(*c*)....	+(*a*)(*b*)(*c*)	−	+	+
9	....	....	....						
10	550	223	3,500	....	+(*a*)(*b*) ...	+(*a*)(*b*)(*c*)	−	−	*x*
11	550	152	2,200	+(*c*).....	+(*b*)(*c*)....	+(*b*)(*c*)...	+	+	+
12	1,200	....	6,650	+(*c*).....	+(*c*).......	+(*c*).......	+	+	+
13	1,000	1,830	800	+(*c*).....	+(*a*)(*b*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
14	600	648	4,700	+(*c*).....	+(*a*)(*b*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
15	490	850	3,550	+(*c*).....	+(*a*)(*b*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
16									
17	700	281	7,000	+(*a*)(*c*)..	+(*b*)(*a*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
18	3,000	630	3,900	+(*a*)(*c*)..	+(*b*)(*a*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
19	1,100	270	1,750	+(*a*)(*b*) .	+(*c*)(*a*)(*b*)	+(*c*).......	−	+	+
20	600	196	830	....	+(*c*).......	+(*b*)(*c*)...	−	+	+
21	450	120	500	....	+(*c*).......	+(*a*)(*b*)(*c*)	−	+	+
22	150	108	470	....	+(*a*)(*b*)...	+(*a*)(*b*)(*c*)	+	+	+
23									
24	4,200	160	10,700	+(*c*).....	+(*a*)(*b*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
25	1,800	500	3,200	+(*a*)(*c*)..	+(*b*)(*a*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
26	2,900	340	3,400	+(*c*).....	+(*b*)(*c*)...	+(*a*)(*b*)(*c*)	+	+	+
27	440	678	1,550	....	+(*a*)......	+(*b*)(*c*)(*a*)	−	−	+
28	300	500	1,900	....	....	....	−	+	+
29	270	100	2,100	....	+(*b*)(*c*)...	+(*b*)(*c*)...	−	+	+
30									
Average	2,324+	483+	4,593+						

Table showing the number of bacteria and presence of colon bacilli found in samples taken from the inlet of Dalecarlia Reservoir—Continued.

JULY.

Date.	Number of bacteria per cubic centimeter.			*B. coli* in—			Fermentation in sugar broth (*c*).		
	(*a*) Filtration plant.	(*b*) Agricultural Department.	(*c*) Hygienic Laboratory.	0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1907.									
uly 1	1,600	131	5,600	+(*a*)(*c*)	+(*a*)(*b*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
2	1,900	84	2,950	+(*c*)	+(*a*)(*b*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
3	1,200	202	150		+(*c*)	+(*b*)(*c*)	+	+	+
4									
5	920	162	350		+(*c*)	+(*c*)	–	+	+
6	370	97	350		+(*c*)	+(*b*)(*c*)	–	+	+
7									
8		132	960		+(*c*)	+(*c*)	–	+	+
9	130	748	2,050	+(*c*)	+(*c*)	+(*b*) (*c*)	+	+	+
10		678	2,100	+(*a*) (*c*)	+(*c*) (*a*)	+(*b*)(*c*)(*a*)	+	+	+
11	470	1,015	1,305		+(*c*)	+(*c*)	–	+	+
12	600	444	1,150	+(*c*)	+(*c*)	+(*b*)(*c*)	+	+	+
13	2,800	433	4,700	+(*a*)(*c*)	+(*a*)(*b*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
14									
15	2,800	189	2,800	+(*a*)	+(*a*)(*b*)(*c*)	+(*a*)(*b*)(*c*)	–	+	+
16		1,100	1,750	+(*b*)	+(*b*)(*c*)	+(*b*)(*c*)	–	+	+
17	500	460	2,150	+(*b*)(*c*)	+(*c*)	+(*b*)(*c*)	+	+	+
18	1,050	253	400	+(*c*)	+(*b*)	+(*b*)(*c*)	+	–	+
19	3,400	401	2,500	+(*c*)	+(*a*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
20	550	125	850	+(*a*)	+(*a*)(*b*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
21									
22	7,400	137	2,400	+(*c*)	+(*a*)(*b*)(*c*)	+(*a*)(*c*)	+	+	+
23	15,400	1,400	1,200	+(*c*)	+(*b*)(*c*)	+(*b*)(*c*)	+	+	+
24		134	950	+(*c*)	+(*b*)	+(*a*)(*b*)(*c*)	+	–	+
25	1,800	148	500	+(*c*)	+(*c*)	+(*c*)	+	+	+
26	410	160	1,050			+(*b*)(*c*)	–	–	+
27	1,040	32	4,400		+(*b*)(*c*)	+(*c*)	–	+	+
28									
29	9,300	22	750	+(*b*)		+(*a*)(*c*)	–	–	+
30		45	550		+(*c*)	+(*a*)(*c*)	–	+	+
31		426	700		+(*c*)	+(*b*)(*c*)	–	+	+
Average	2,682	352+	1,715+						

Table showing the number of bacteria and presence of colon bacilli found in samples taken from the inlet of Dalecarlia Reservoir—Continued.

AUGUST.

Date.	Number of bacteria per cubic centimeter.			*B. coli* in—			Fermentation in sugar broth (*c*).		
	(*a*) Filtration plant.	(*b*) Agricultural Department.	(*c*) Hygienic Laboratory.	0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1907.									
Aug. 1	....	35	3,000	....	....	+(*a*) (*b*)...	–	–	–
2	12,200	92	180	....	....	+(*a*)......	–	–	–
3	220	46	....	....	....	....	....	....	....
4	....	....	....	....	....	....	....	....	....
5	720	26	400	....	+(*c*)......	+(*c*)......	–	+	+
6	2,500	21	200	....	....	+(*a*) (*b*) (*c*)	–	–	+
7	1,300	112	220	....	+(*a*)......	+(*a*) (*b*)...	–	+	+
8	2,300	49	200	....	....	....	–	–	+
9	2,200	65	260	....	....	+(*b*)......	–	–	–
10	31,400	63	50,000	+ (*a*) (*c*)	+(*a*) (*c*)...	+(*a*) (*b*) (*c*)	+	+	+
11	....	....	....	....	....	....	....	....	....
12	2,800	48	25,000	....	+(*a*) (*b*) (*c*)	+(*a*) (*b*) (*c*)	+	+	+
13	760	45	2,450	....	+(*b*) (*c*)...	+(*b*) (*c*)...	+	+	+
14	4,500	133	16,900	....	+(*a*) (*c*)...	+(*a*) (*c*)...	–	+	+
15	1,200	200	700	....	....	+(*a*)......	–	+	+
16	550	50	300	+(*c*)....	+(*a*) (*c*)...	+(*a*) (*c*)...	+	+	+
17	310	127	330	....	+(*b*)......	+(*b*)......	–	–	+
18	....	....	....	....	....	....	....	....	....
19	650	187	400	....	+(*b*) (*c*)...	+(*b*) (*c*)...	–	+	+
20	240	23	110	+ (*c*)...	....	+(*a*) (*c*)...	+	–	+
21 *	1,300	895	850	....	....	+(*b*) (*c*)...	–	+	+
22	3,800	790	1,110	+ (*c*)...	+(*b*) (*c*)...	+(*b*) (*c*)...	+	+	+
23 ‡	2,500	306	2,250	+ (*c*)...	+(*c*)......	+(*c*)......	+	+	+
24	3,900	350	1,400	....	+(*c*)......	+(*b*) (*c*)...	+	+	+
25	....	....	....	....	....	....	....	....	....
26	700	190	680	+ (*c*)...	+(*a*) (*b*) (*c*)	+(*a*) (*b*) (*c*)	+	+	+
27	470	....	340	....	+(*a*) (*c*)...	+(*a*) (*c*)...	–	+	+
28	500	....	230	....	....	+(*c*)......	–	+	+
29	360	....	....	....	+(*c*)......	+(*c*)......	–	+	+
30	320	....	....	....	+(*a*) (*c*)...	+(*a*) (*c*)...	–	+	+
31	200	115	200	....	+(*c*)......	+(*c*)......	–	+	+
Average....	2,957+	172+	4,487+						

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* Dalecarlia reservoir by-passed from Aug. 21 to Dec. 31.

‡ From this date samples taken from the inlet of Georgetown reservoir.

able showing the number of bacteria and presence of colon bacilli found in samples taken from the inlet of Dalecarlia Reservoir—Continued.

SEPTEMBER.

Date.	Number of bacteria per cubic centimeter			*B. coli* in—			Fermentation in sugar broth (*c*).		
	(*a*) Filtration plant.	(*b*) Agricultural Department.	(*c*) Hygienic Laboratory.	0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1907.									
ept. 3	300	106	160	....	....	+(*c*)	–	–	+
4	600	208	250	....	+ (*a*) (*c*)	+(*a*)(*c*)	–	+	+
5	360	....	550	....	....	+(*c*)	–	+	+
6	15,000	....	840	+(*c*)	+ (*a*) (*c*)	+(*a*)(*c*)	+	+	+
7	2,000	382	700	+(*c*)	+(*b*)(*c*)	+(*a*)(*b*)	+	+	+
8	....	....	....	....	....	....	....	....	....
9	220	300	....	....	+(*b*)(*c*)	+(*b*)(*c*)	–	+	+
10	18,000	530	....	....	+(*a*)(*b*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
11	2,700	340	800	+(*a*)(*c*)	+(*a*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
12	1,000	716	800	+(*c*)	+(*a*)(*b*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
13	2,300	510	860	....	+(*a*)(*b*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
14	2,400	566	500	+(*b*)	+(*a*)(*b*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
15	....	....	....	....	....	....	....	....	....
16	....	430	550	....	+(*b*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
17	420	....	1,250	....	+(*a*)	+(*a*)(*c*)	–	–	+
18	900	....	570	....	+(*c*)	+(*c*)	–	+	+
19	2,000	1,660	1,700	....	+(*b*)(*c*)	+(*b*)(*c*)	–	+	+
20	4,200	1,200	1,800	....	+(*b*)(*c*)	+(*b*)(*c*)	+	+	+
21	1,100	550	1,150	....	+(*c*)	+(*c*)	+	+	+
22	....	....	....	....	....	....	....	....	....
23	2,400	....	1,100	....	....	+(*c*)	+	+	+
24	4,000	....	1,300	....	+(*c*)	+(*c*)	+	+	+
25	56,000	4,000	1,080	+(*a*)(*b*)	+(*a*)(*b*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
26	13,000	2,625	5,000	+(*a*)(*b*)	+(*a*)(*b*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
27	4,000	850	4,300	+(*a*)(*b*)(*c*).	+(*a*)(*b*)(*c*)	+(*a*)(*c*)	+	+	+
28	15,000	865	4,200	+(*a*)	+(*a*)(*b*)(*c*)	+(*a*)(*c*)	+	+	+
29	....	....	....	....	....	....	....	....	....
30	....	3,500	12,800	+(*a*)(*c*)	+(*a*)(*b*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
Average	6,722+	590+	1,920+						

Table showing the number of bacteria and presence of colon bacilli found in samples take from the inlet of Dalecarlia Reservoir—Continued.

OCTOBER.

Date.	Number of bacteria per cubic centimeter.			*B. coli* in—			Fermentation in sug broth (*c*).		
	(*a*) Filtration plant.	(*b*) Agricultural Department.	(*c*) Hygienic Laboratory.	0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c.
1907.									
Oct. 1	600	550	6,000	+(*c*)	+(*a*)(*b*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
2	4,400	285	3,200	+(*c*)	+(*b*)(*c*)	+(*b*)(*c*)	+	+	+
3	900	300	875	+(*c*)	+(*a*)(*b*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
4	850	320	550	+(*a*)(*c*)	+(*a*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
5	2,000	285	510	+(*c*)	+(*a*)(*b*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
6									
7	1,250	65	440	+(*c*)	+(*a*)(*b*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
8	11,000	118	606	....	+(*a*)(*b*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
9	2,000	170	970	....	+(*a*)(*b*)(*c*)	+(*a*)(*b*)(*c*)	–	+	+
10	800	2,500	400	....	....	+(*b*)(*c*)	+	+	+
11	2,000	395	390	+(*a*)(*c*)	+(*a*)(*b*)(*c*)	+(*a*)(*c*)	+	+	+
12	1,200	203	260	+(*c*)	+(*a*)(*b*)(*c*)	+(*a*)(*b*)(*c*)	+	+	+
13									
14	1,200	252	340	....	+(*c*)	+(*b*)(*c*)	–	+	+
15	900	....	660	....	+(*a*)(*c*)	+(*a*)(*c*)	–	+	+
16	....	290	3,550	....	+(*a*)(*b*)	+(*a*)(*b*)(*c*)	–	–	+
17	550	150	510	....	....	+(*a*)((*b*)(*c*)	–	–	+
18	260	204	790	....	+(*b*)(*c*)	+(*b*)(*c*)	–	+	+
19	750	280	730	....	+(*c*)	+(*c*)	–	+	+
20									
21	480	180	1,050	....	+(*c*)	+(*b*)(*c*)	–	+	+
22	230	108	850	....	....	....	+	+	+
23	250	190	480	....	....	....	–	–	+
24	300	80	1,400	....	+(*b*)	+(*c*)	–	–	+
25	450	50	480	....	....	+(*b*)(*c*)	–	–	+
26	450	74	350	....	....	+(*a*)(*c*)	–	+	+
27									
28	190	202	550	....	....	....	–	–	–
29	380	320	1,325	....	+(*c*)	+(*c*)	–	+	+
30	450	100	500	....	....	+(*b*)	–	–	+
31	2,300	1,100	2,800	....	+(*c*)	+(*a*)(*b*)(*c*)	–	+	+
Average	1,390	337+	1,132+						

Table showing the number of bacteria and presence of colon bacilli found in samples taken from the inlet of Dalecarlia Reservoir—Continued.

NOVEMBER.

Date.	Number of bacteria per cubic centimeter.			*B. coli* in—			Fermentation in sugar broth (*c*).		
	(*a*) Filtration plant.	(*b*) Agricultural Department.	(*c*) Hygienic Laboratory.	0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1907.									
Nov. 1	550	750	3,775		+(*c*)	+(*a*) (*c*)	−	+	+
2	900	146	800			+(*a*) (*c*)	−	−	+
3									
4	5,500	10,000	19,000	+(*a*) (*c*)	+(*a*) (*c*)	+(*a*) (*c*)	+	+	+
5	6,000	40,000	20,000	+(*a*) (*c*)	+(*a*) (*b*) (*c*)	+(*a*) (*b*) (*c*)	+	+	+
6	5,000	13,000	14,700	+(*c*)	+(*a*) (*b*) (*c*)	+(*a*) (*c*)	+	+	+
7	14,000	5,000	56,000		+(*a*) (*c*)	+(*a*) (*c*)	−	+	+
8	1,900	4,500	12,300	+(*a*)	+(*a*) (*c*)	+(*a*) (*c*)	−	+	+
9	4,000	6,000	7,900	+(*a*) (*c*)	+(*a*) (*c*)	+(*a*) (*c*)	+	+	+
10									
11	1,200	1,100	7,200			+(*c*)	−	+	+
12	7,500	1,850	2,600		+(*a*) (*c*)	+(*a*) (*c*)	−	+	+
13	1,600	1,140	3,100		+(*a*)	+(*a*) (*c*)	−	+	+
14	2,700	1,080	2,650		+(*c*)	+(*a*) (*b*) (*c*)	+	+	+
15	1,800	800	2,100			+(*a*) (*c*)	−	+	+
16	1,100		1,900	+(*c*)	+(*c*)	+(*c*)	+	+	+
17									
18	1,600	1,400	2,250	+(*c*)	+(*c*)	+(*c*)	+	+	+
19	1,300	1,500	1,700		+(*c*)		−	+	−
20	500		11,800	+(*c*)	+(*a*) (*c*)	+(*a*) (*c*)	+	+	+
21	9,900		18,800	+(*c*)	+(*a*) (*c*)	+(*a*) (*c*)	+	+	+
22	10,000		13,700	+(*c*)	+(*a*) (*c*)	+(*a*) (*c*)	+	+	+
23	18,000		24,500	+(*a*)	+(*a*) (*c*)	+(*a*) (*c*)	+	+	+
24									
25	50,000		14,200	+(*a*) (*c*)	+(*a*) (*c*)	+(*a*) (*c*)	+	+	+
26	40,000		36,000	+(*c*)	+(*a*) (*c*)	+(*a*) (*c*)	+	+	+
27	16,000		74,000	+(*c*)	+(*a*) (*c*)	+(*a*) (*c*)	+	+	+
28									
29	10,000		139,600		+(*a*) (*c*)	+(*a*) (*c*)	−	+	+
30	3,800		10,800			+(*c*)	−	+	+
Average	9,634	5,885+	20,055−						

THE FILTERED RIVER WATER.

Table showing the number of bacteria and presence of colon bacilli in samples taken from the outlet of the filtered water reservoir.

[Daily examinations made by the (*a*) Laboratory of the filtration plant; (*b*) Bureau of Plant Industry of Agricultural Department; (*c*) hygienic laboratory, Public Health and Marine-Hospital Service.]

MAY.

Date.	Number of bacteria per cubic centimeter.			*B. coli* in—			Fermentation in sugar broth (*c*).		
	(*a*) Filtration plant.	(*b*) Agricultural Department.	(*c*) Hygienic Laboratory.	0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1907.									
May 15	21	3	24					—	
16	13	12	29					—	
17	35	10	33					—	
18	16	3	22					—	
19									
20	24	16	25					—	
21	27	13	28					—	
22	28	6	29					—	
23	20	9	24					—	
24	20	15	28					—	
25	39	12	20					—	
26									
27	25		21					—	
28	39	13	14					—	
29	35		16					—	
30									
31	32	5	14			+ (*c*)		—	+
Average	26+	9+	23+						

Table showing the number of bacteria and presence of colon bacilli in samples taken from the outlet of the filtered water reservoir—Continued.

JUNE.

Date.	Number of bacteria per cubic centimeter.			*B. coli* in—			Fermentation in sugar broth (*c*).		
	(*a*) Filtration plant.	(*b*) Agricultural Department.	(*c*) Hygienic Laboratory.	0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1907.									
June 1	24	5	(?)	...	...	...	...		...
2	...	...	...	...	...	...	...	...	...
3	28	4	18	...	...	...	...	—	...
4	23	9	16	...	...	...	...	—	...
5	24	7	11	...	...	...	...	—	...
6	10	14	32	...	...	...	...	—	...
7	13	30	18	...	...	...	...	—	...
8	22	11	(?)	...	...	...	...	—	...
9	...	...	...	...	...	...	...	—	...
10	16	20	39	...	...	...	...	—	...
11	21	13	70	...	...	...	...	—	...
12	16	14	39	...	...	...	...	—	...
13	24	300	35	...	...	...	...	—	+
14	34	17	25	...	...	...	...	—	...
15	10	400	27	...	...	...	...	—	...
16	...	...	...	...	...	...	...	...	...
17	30	16	35	...	...	...	...	—	...
18	21	19	38	...	...	...	...	—	...
19	20	15	23	...	...	...	...	—	...
20	9	9	(?)	...	...	...	...	—	...
21	17	21	22	...	...	...	...	—	...
22	8	19	22	...	...	...	...	—	...
23	...	...	...	...	...	...	...	...	...
24	19	11	26	...	...	...	...	—	...
25	10	14	26	...	...	...	...	—	...
26	21	30	37	...	...	...	...	—	...
27	13	10	54	...	...	...	...	—	+
28	11	134	(?)	...	...	...	...	—	...
29	13	4	22	...	...	...	...	—	...
30	...	...	...	...	...	...	...	...	...
Average	18+	45+	29+						

Table showing the number of bacteria and presence of colon bacilli in samples taken from the outlet of the filtered water reservoir—Continued.

JULY.

Date.	Number of bacteria per cubic centimeter.			*B. coli* in—			Fermentation in sugar broth (*c*).		
	(*a*) Filtration plant.	(*b*) Agricultural Department.	(*c*) Hygienic Laboratory.	0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1907.									
July 1	14	17	27	....	....	....	....	—	....
2	11	30	33	....	....	....	....	—	....
3	....	23	36	....	....	....	....	—	....
4	22	....	....	....	....	....	....	....	....
5	32	11	34	....	....	....	....	—	....
6	33	8	16	....	....	....	....	—	....
7	....	....	....	....	....	....	....	....	....
8	20	12	54	....	....	....	....	—	....
9	8	3	32	....	....	....	....	—	....
10	....	90	21	....	....	....	....	—	....
11	7	13	36	....	....	....	....	—	....
12	7	16	22	....	....	....	....	—	+
13	12	13	24	....	....	....	....	—	....
14	12	....	....	....	....	....	....	....	....
15	21	....	34	....	....	....	....	—	....
16	19	100	20	....	....	....	....	—	....
17	6	17	70	....	....	....	....	—	....
18	9	13	49	....	....	....	....	—	+
19	19	22	28	....	....	....	....	—	....
20	....	8	41	....	....	....	....	—	....
21	....	....	....	....	....	....	....	....	....
22	21	46	29	....	....	....	....	—	....
23	25	....	30	....	....	....	....	—	....
24	20	7	33	....	....	....	....	—	....
25	14	63	35	....	....	....	....	—	....
26	11	15	24	....	....	....	....	—	....
27	17	16	24	....	....	....	....	—	....
28	18	....	....	....	....	....	....	....	....
29	23	2	34	....	....	....	....	—	....
30	....	6	34	....	....	....	....	—	....
31	....	15	114	....	....	....	....	—	....
Average	16+	24+	36+						

Table showing the number of bacteria and presence of colon bacilli in samples taken from the outlet of the filtered water reservoir—Continued.

AUGUST.

Date.	Number of bacteria per cubic centimeter.			*B. coli* in—			Fermentation in sugar broth (*c*).		
	(*a*) Filtration plant.	(*b*) Agricultural Department.	(*c*) Hygienic Laboratory.	0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1907.									
Aug. 1	9	10	28	....	....	....	....	—	....
2	6	37	28	....	....	....	....	—	....
3	18	2	....	....	....	....	....	....	....
4	20	....	....	....	....	....	....	....	....
5	15	5	26	....	....	....	....	—	....
6	19	3	22	....	....	....	....	—	....
7	27	7	(?)	....	....	....	....	—	....
8	8	16	19	....	....	....	....	—	....
9	8	8	46	....	....	....	....	—	....
10	9	....	36	....	....	....	....	—	....
11	17	....	....	....	....	....	....	....	....
12	10	2	34	....	....	+(*c*)	....	—	+
13	7	11	47	....	....	....	....	—	....
14	14	17	54	....	....	....	....	—	....
15	10	20	44	....	....	....	....	—	....
16	10	15	30	....	....	....	....	—	....
17	24	74	40	....	....	....	....	—	....
18	32	....	....	....	....	....	....	....	....
19	21	15	32	....	....	....	....	—	....
20	27	12	34	....	....	....	....	—	+
21 [a]	21	21	39	....	....	....	....	—	+
22	20	3	35	....	....	....	....	—	....
23	18	22	45	....	....	....	....	—	....
24	34	15	50	....	....	....	....	—	....
25	22	....	....	....	....	....	....	....	....
26	25	13	35	....	....	....	....	—	....
27	10	....	32	....	....	....	....	—	....
28	5	....	26	....	....	....	....	—	....
29	9	....	(?)	....	....	....	....	—	....
30	25	....	(?)	....	....	....	....	—	....
31	12	....	46	....	....	....	....	—	....
Average	16+	15+	34+						

[a] Dalecarlia Reservoir by-passed from Aug. 21.

Table showing the number of bacteria and presence of colon bacilli in samples taken from the outlet of the filtered water reservoir—Continued.

SEPTEMBER.

Date.	Number of bacteria per cubic centimeter.			*B. coli* in—			Fermentation in sugar broth (*c*).		
	(*a*) Filtration plant.	(*b*) Agricultural Department.	(*c*) Hygienic Laboratory.	0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1907.									
Sept. 1	65	....	....	....	....	....	....	....	....
2	39	....	....	....	....	....	....	....	....
3	36	22	....	....	....	....	....	—	....
4	23	7	52	....	....	....	....	—	....
5	51	....	65	....	....	+ (*c*)	....	—	+
6	38	....	54	....	....	....	....	—	+
7	34	5	90	....	....	....	....	—	....
8	33	....	....	....	....	....	....	....	....
9	30	7	(?)	....	....	....	....	—	....
10	30	6	80	....	....	....	....	—	+
11	20	6	30	....	....	....	....	—	....
12	25	5	30	....	....	....	....	—	+
13	62	13	58	....	....	+(*a*)	....	—	....
14	42	5	23	....	....	....	....	—	....
15	70	....	....	....	....	....	....	....	....
16	23	6	100	....	....	....	....	—	....
17	10	....	26	....	....	....	....	—	....
18	9	....	31	....	....	....	....	—	....
19	6	12	23	....	....	....	....	—	+
20	....	11	41	....	....	....	....	—	....
21	19	7	22	....	....	....	....	—	....
22	20	....	....	....	....	....	....	....	....
23	18	....	140	....	....	....	....	—	....
24	190	....	70	....	....	....	....	—	+
25	24	2	48	....	....	....	....	—	....
26	47	1	190	....	....	....	....	—	....
27	19	8	180	....	....	+ (*c*)	....	—	+
28	24	500	190	....	....	+ (*c*)	....	+	+
29	55	....	....	....	....	....	....	....	....
30	....	10,000	220	....	+ (*b*) (*c*)..	+ (*b*) (*c*)..	....	+	+
Average	37+	590+	80+						

Table showing the number of bacteria and presence of colon bacilli in samples taken from the outlet of the filtered water reservoir—Continued.

OCTOBER.

Date.	Number of bacteria per cubic centimeter.			*B. coli* in—			Fermentation in sugar broth (*c*).		
	(*a*) Filtration plant.	(*b*) Agricultural Department.	(*c*) Hygienic Laboratory.	0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1907.									
Oct. 1	42	12	260	....	....	....	....	+	+
2	220	45	240	....	+ (*b*) (*c*)..	+ (*b*) (*c*)..	....	+	+
3	31	10	56	....	....	+ (*b*)	....	—	+
4	38	14	56	....	....	+ (*a*) (*b*) (*c*)	....	+	+
5	44	23	22	....	....	+ (*b*) (*c*)..	....	—	+
6	30	....	....	....	....	+ (*a*)	....	....	....
7	40	3	34	....	....	+ (*b*) (*c*)..	....	—	+
8	24	4	28	....	....	+ (*b*) (*c*)..	....	—	+
9	30	3	70	....	....	+ (*b*)	....	—	—
10	26	3	18	....	....	....	....	—	—
11	44	4	120	....	....	....	....	—	—
12	75	....	100	....	....	+ (*c*)	....	—	+
13	38	....	....	....	....	....	....	....	....
14	43	10	28	....	....	....	....	—	....
15	50	....	40	....	....	....	....	—	....
16	13	9	20	....	....	....	....	—	....
17	14	10	100	....	....	....	....	—	....
18	19	9	18	....	....	....	....	—	+
19	21	6	16	....	....	+ (*c*)	....	—	+
20	32	....	....	....	....	....	....	....	....
21	28	5	60	....	....	....	....	—	—
22	13	4	39	....	....	....	....	—	—
23	11	....	180	....	....	....	....	—	—
24	14	6	500	....	....	....	....	—	—
25	19	6	140	....	....	....	....	—	—
26	18	9	20	....	....	....	....	—	—
27	....	....	....	....	....	....	....	....	....
28	15	6	30	....	....	....	....	—	—
29	10	7	30	....	....	....	....	—	....
30	4	2	70	....	....	....	....	—	....
31	8	9	18	....	....	....	....	—	....
Average	41+	9+	84+						

Table showing the number of bacteria and presence of colon bacilli in samples taken from the outlet of the filtered water reservoir—Continued.

NOVEMBER.

Date.	Number of bacteria per cubic centimeter.			*B. coli* in—			Fermentation in sugar broth (*c*).		
	(*a*) Filtration plant.	(*b*) Agricultural Department.	(*c*) Hygienic Laboratory.	0.1 c. c.	1 c. c.	10 c. c.	0.1 c. c.	1 c. c.	10 c. c.
1907.									
Nov. 1	18	27	130					–	
2	6	7	21					–	
3	17								
4	12	7	21			+ (*c*)		–	+
5	10	6	15					–	
6	12	6	17					–	
7		5	59					–	+
8	17	10	30					–	
9	21	8	14					–	
10	25								
11	23	3	14					–	
12	22	8	19					–	
13	17	4	33					–	
14	10	8	30					–	
15	17	6	100					–	+
16	28		68					+	+
17	20								
18	17	11	47			+ (*c*)		–	+
19	16	12	98					–	–
20	12		33					–	–
21	15		25					–	–
22	20		44					–	+
23	21		36			+ (*c*)		–	+
24	55								
25	48		66					+	–
26	43		155					–	–
27	60		103			+ (*c*)		+	+
28	70								
29	70		185					–	+
30	60		216					–	+
Average	26+	8+	63+						

These results show the great extent of bacterial purification which the water underwent in its passage through the storage reservoirs and filter beds.

The effluent from the filter beds during the period extending from May 15 to August 31 can not be regarded from a bacteriological standpoint other than as a water of good sanitary quality. During this period, of the 84 samples of which counts were made by us, the average number of bacteria per cubic centimeter was about 31. Ninety-one samples were examined in quantities of 0.1 c. c., 1 c. c., and 10 c. c., for the presence of the colon bacillus. In none of the 0.1 c. c. and 1 c. c. quantities was *B. coli* found, and of the 10 c. c. quantities, *B. coli* was found in only 2, or about 2.2 per cent.

In 1906 the samples of water, taken at intervals of a few days during the period from July 25 to August 31, from the filtered water reservoir, and examined by us, showed an average of 44 bacteria per cubic centimeter. Of 13 samples examined in this period, *B. coli* was found in 1 c. c. once and in 10 c. c. twice. Unfortunately the number of samples from the filtered water reservoir examined by us in 1906 was too small to compare aptly with the results of 1907, but during the period extending from July 16 to August 31, 1906, we examined 60 samples of tap water taken from taps in various parts of the city. During this period the average number of bacteria per cubic centimeter in the tap water was about 67, and of the 60 samples, *B. coli* was found in quantities of 1 c. c. twice, and in quantities of 10 c. c. sixteen times, or in about 3.3 per cent of the 1 c. c. samples and in about 26.6 per cent of the 10 c. c. samples.

It will be observed in the above tables that the remarkably high degree of purity of the water accomplished by the storage and filtration in the summer of 1907 was not maintained during September and October. According to our own results, the total bacterial content of the filtered water during September averaged 80 per cubic centimeter, and during October, 84 per cubic centimeter. In September, of the 1 c. c. samples examined, *B. coli* was found in 4.3 per cent and of the 10 c. c. samples in 17.4 per cent. In October, *B. coli* was found in 3.6 per cent of the 1 c. c. samples and in 26 per cent of the 10 c. c. samples examined. In this connection it is interesting to note that Dalecarlia, the principal sedimentation reservoir, was by-passed from August 21 to December 31, 1907.

It should be stated that at present the generally accepted view is that the colon bacillus is only an index of pollution. It suggests danger, but not the amount of danger. In other words, a water may contain a large number of colon bacilli and yet be entirely free of typhoid bacilli.

In November the average bacterial content of the filtered water was 63 per cubic centimeter, and *B. coli* was found in 16 per cent of the 10 c. c. samples examined. The low bacterial content of the filtered water in November is interesting when considered in connection with the fact that the raw river water during this month averaged (our results) over 15,000 bacteria per cubic centimeter more than it did in either September or October.

According to bacteriological standards of pure water, the water delivered through the filtration plant to the city of Washington during May, June, July, and August of 1907 was of a high degree of purity, and on this evidence alone it would appear justifiable to exclude this water as a factor in the conveyance of typhoid fever infection.

The filtered water this summer was of a better quality than it was in the summer of 1906; that is, as nearly as we can judge by our bacteriological results of this summer in comparison with those of our more limited studies in 1906. The improvement in the water being followed by the lower rate of prevalence of typhoid fever in the summer of 1907 suggests cause and effect. The known factors, other than water, seemed, so far as the results of our epidemiological studies indicate, to have operated relatively to even a less extent during the summer of 1906 than in that of 1907. This suggests that the difference in morbidity rate of typhoid fever for the two summers was due to a difference in the amount of the water-borne typhoid fever infection.

However, it should be borne in mind that the prevalence of typhoid fever in Washington and other communities has varied considerably in different years during which the conditions as to water, etc., have, so far as known, remained approximately the same.

Further, it should be stated that the improvement in the quality of the Potomac River water accomplished by sand filtration in 1906 was not followed by a lowering of the typhoid fever rate. In cities where infected water is known to have played a conspicuous rôle, the rule has been that the filtration or improvement of the water supply has been followed by an immediate impress upon the height of the typhoid curve. In Paterson, N. J., however, the decline in the rate was not apparent until the second year following the filtration of the public water supply. At Lawrence, Mass., the filtration of the city water supply was followed the first year by a marked decrease, but it required three years to produce its maximum effect. There has been much discussion in regard to the fact that in 1906 the improvement in the Potomac River water by filtration was not followed by an apparent lowering of the typhoid fever rate in Washington. The view is held by some that this fact strongly indicates that little if any of the typhoid fever in Washington was ever conveyed in the Potomac water. In this connection it is interesting to note that according to our results and those obtained in the laboratory at the filtration plant in 1906, from 55 to 86 per cent of the total bacteria in the Potomac River water are removed by storage in the three sedimentation reservoirs before the water is applied to the filter beds. These storage reservoirs have been in operation since 1902. During the two or three years prior to the installation of the filters no systematic bacteriologic examination of the water was made; so we do not know what was the bacterial content of the water supplied to Washington during that period and can not accurately compare it with that of the water after filtration was begun. We have no accurate data on which to base a comparison of the amount of typhoid fever infected excreta poured into the Potomac River in 1906 with that of previous years. We know practically nothing of the condition of symbiosis, etc., under which the typhoid bacillus will live best in a river water during certain seasons and perhaps certain years.

The quite regular seasonal (summer) prevalence of typhoid fever in Washington, together with the absence of frequent winter and spring outbreaks, is regarded by some as good evidence that the Potomac River water has played but a minor rôle in transmitting the infection. In Albany and other cities where contaminated water is known to have played a prominent rôle, improvement in the water supply not only diminished the total amount of typhoid fever, but changed the character of the typhoid curve. The rule is that where there is much water-borne typhoid there is also comparatively much winter and spring typhoid. In Albany the filtration of the water markedly reduced the prevalence of typhoid fever throughout the entire year, but most markedly during the winter and spring, during which seasons there was, prior to filtration, relatively much more typhoid than during the summer season. On the other hand, Washington has had relatively little winter and spring typhoid; the annual curve, even when excessively high and before any attempted purification of the water supply, shows a marked summer maximum or the so-called "normal" curve. The Washington curve has changed markedly in height, but little in character.

In the majority of cities supplied with a polluted water and having a high typhoid rate in the winter and spring, the pollution of the water has been nearer the intake and presumably much more concentrated than is the case of Washington's water supply. The vast bulk of the pollution of the Potomac occurs at points over 100 miles above the intake of the water supply for Washington, and little or practically no direct pollution takes place within 19 miles of the intake. Therefore, as the dilution of the sewage must be tremendous by the time it reaches the intake at Great Falls it may be that the small amount of typhoid infection finding its way into the river from the relatively small number of cases occurring on the watershed during the winter and early spring is not sufficient to affect markedly the prevalence of the disease in Washington, but with the increase of cases on the watershed in the summer there may be, in spite of the dilution, enough typhoid infection in the water to occasion a marked increase in the prevalence of the disease in Washington. In this connection reference should be made to Kober's observations on the effects of the typhoid epidemic of 1889–1890 at Cumberland, Md., upon the prevalence of the disease in Washington.[a]

We have noted that in dry and warm seasons the present system of storage and filtration of the Potomac River water will give a water of good sanitary quality, but in periods of high turbidity, especially in winter, fine silt in the river water does not settle so well, nor is it all retained by the sand filters. With the increased amount of this silt in the effluent is an increased number of bacteria.

MILK.

The 523 cases considered as having contracted the infection in the District of Columbia gave the following history as to the use of milk during the thirty days prior to illness:

As a beverage	290
On fruits or cereals, but not as a beverage	118
In hot tea or coffee only	44
As ice cream only	28
In no way	38
Not determined	5
Total	523

Of the cases using milk as a beverage or on fruits and cereals 6 used boiled milk exclusively.

One distinct milk outbreak occurred. This was among the customers of milk dealer No. 163. There were in the course of this outbreak 31 cases with the onset of illness between June 8 and August 17. (See Chart No. 4.) Of these cases 2 gave a history of having used boiled milk only during the thirty days prior to onset, and for one other infection by contact seemed more probable. Eliminating these 3 cases there are left 28, considered as being quite definitely attributable to milk infection.

The evidence that these cases were due to milk infection was not absolutely positive, but it seemed sufficiently strong to justify attributing these cases to milk. Thus, there was a sudden and marked increase in the number of cases among the customers of this dealer. The number of cases among his customers was far in excess of the rate of prevalence among persons living in the same neighborhoods and under practically similar conditions but obtaining milk from other sources.

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[a] Report on the pollution of rivers, by Henry Talbott, Chairman of Committee on River Pollution, to the Game and Fish Protection Association of the District of Columbia. Government Printing Office, Washington, 1898, p. 32.

There were several instances of two or more cases in the same family with onsets on the same day. About 200 families obtained milk from this dairy, and estimating the number of persons in a family at 5, there were about 1,000 persons exposed to this milk infection. The histories obtained as to the ways in which milk was used by the 28 cases during the thirty days prior to the onset of illness were as follows:

	Cases.
As a beverage	20
On fruits and cereals, but not as a beverage.	6
In tea or coffee only	2
Total	28

The ages of the cases were as follows:

Years:	Cases.
0–4	4
5–9	5
10–14	2
15–19	3
20–24	7
25–29	4
30–34	2
40–44	1
Total	28

There were six instances in which the person affected was the only member of the family who used milk freely as a beverage, the other members using it chiefly in fruits, cereals, and coffee. On the other hand, and showing how misleading the exceptional case may be, there was one instance of three children in a family all of whom used milk as a beverage, but the child who drank milk in smallest quantity was the one affected.

In another family there were three children, aged about 2, 4, and 6 years, all of whom used milk as a beverage. For the youngest child the milk was sterilized. This child remained well, while the two who drank the raw milk had typhoid fever, being stricken on about the same date.

The dairy was supplied with milk by two dairy farms. At the beginning of the outbreak one of these farms was inspected by representatives of the health office. No typhoid fever cases were found on this farm; but on account of general conditions the sale of milk from it in the District of Columbia was discontinued by order of the health officer on July 10.

The outbreak continued after an interval more than covering the usual incubation period of typhoid fever (in cases of milk infection) had elapsed after the discontinuance of milk from this farm. So it seems that the bulk of the infection did not come in the milk from this farm.

The inspection of the dairy showed nothing immediate to account for the infection. Three of the men who worked at the dairy gave a history of having had typhoid fever within four to twenty years previous. Specimens of their stools and urine were examined at the Hygienic Laboratory, but all were negative for *B. typhosus.*

The sale of milk from this dairy was discontinued by order of the health officer on August 6.

One of us, conjointly with a representative of the health office, made an inspection of the other farm supplying this dairyman with milk, and the evidence obtained indicated quite strongly that this farm was the source of the infection for the majority of the cases. The owner of this farm did most of the handling of the milk. He was taken ill about July 1 with diarrhea and fever and confined to bed for a day or two. For about five weeks from that time he was complaining, having to lie down for an hour or two most every day; had slight fever, which he thought was malarial. He was engaged in handling the milk nearly every day throughout his illness.

In the neighborhood of this dairy farm were seven families which obtained milk from the farm. In one of these families a child was taken ill about June 8 and continued ill with fever for about four weeks. Milk bottles from the dairy farm were delivered to and collected from this house daily throughout the child's illness.

In another of these families were three cases, clinically typical cases of typhoid fever, having the onset of illness in the early part of August.

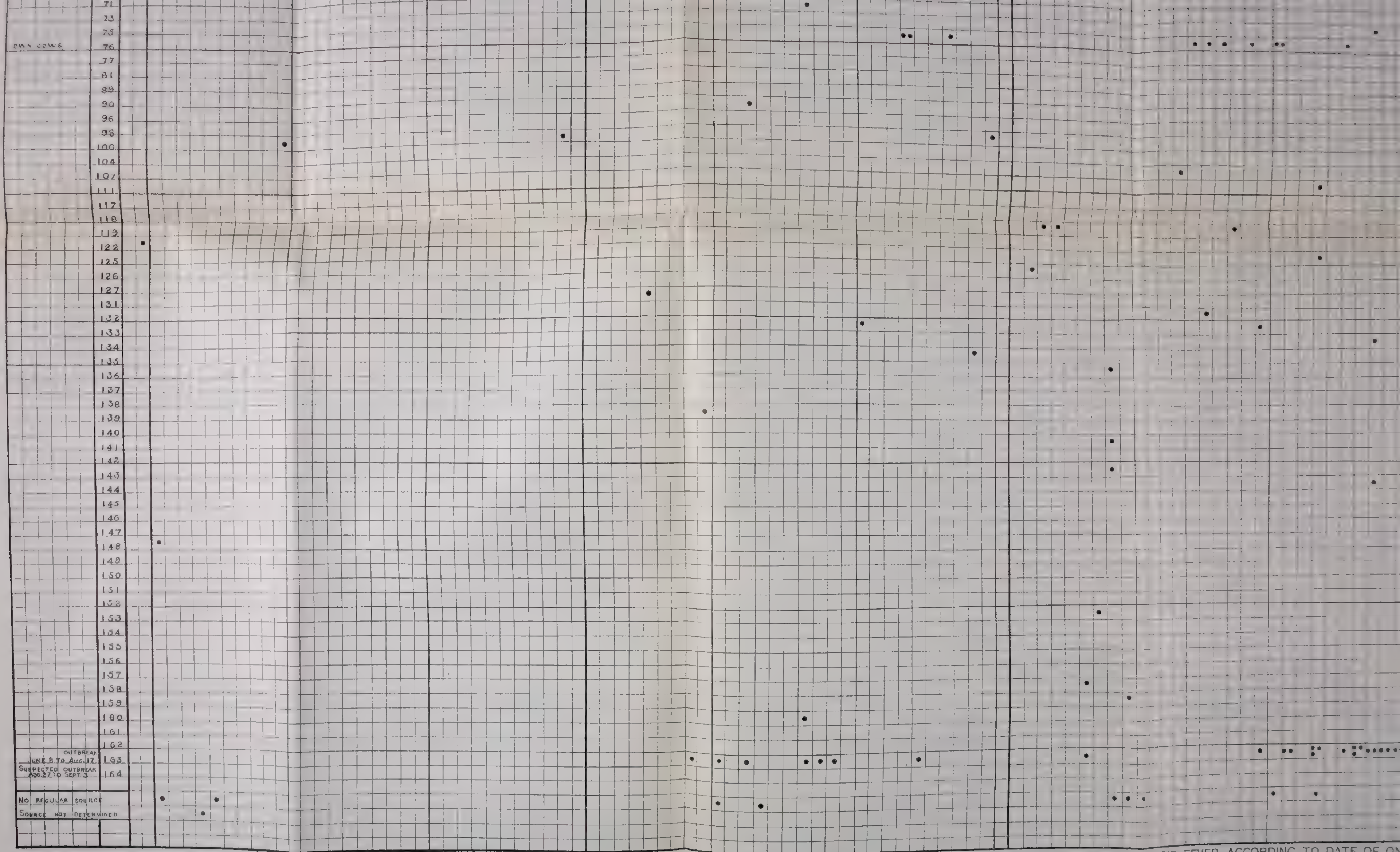
These five cases all occurred in the State of Maryland and are not included in our figures.

The sale of milk from this farm was discontinued by order of the health officer on August 14.

Besides this pronounced outbreak there were suggestive groups of cases on the routes of milk dealers as follows (see Chart No. 4):

	Cases.
Dealer No. 8: Onset of cases from August 5 to September 7	15
Dealer No. 46: Onset of cases from August 14 to September 4	13
Dealer No. 164: Onset of cases from August 27 to September 5	4
Dealer No. 44: Onset of cases from August 9 to 26	8
Total	40

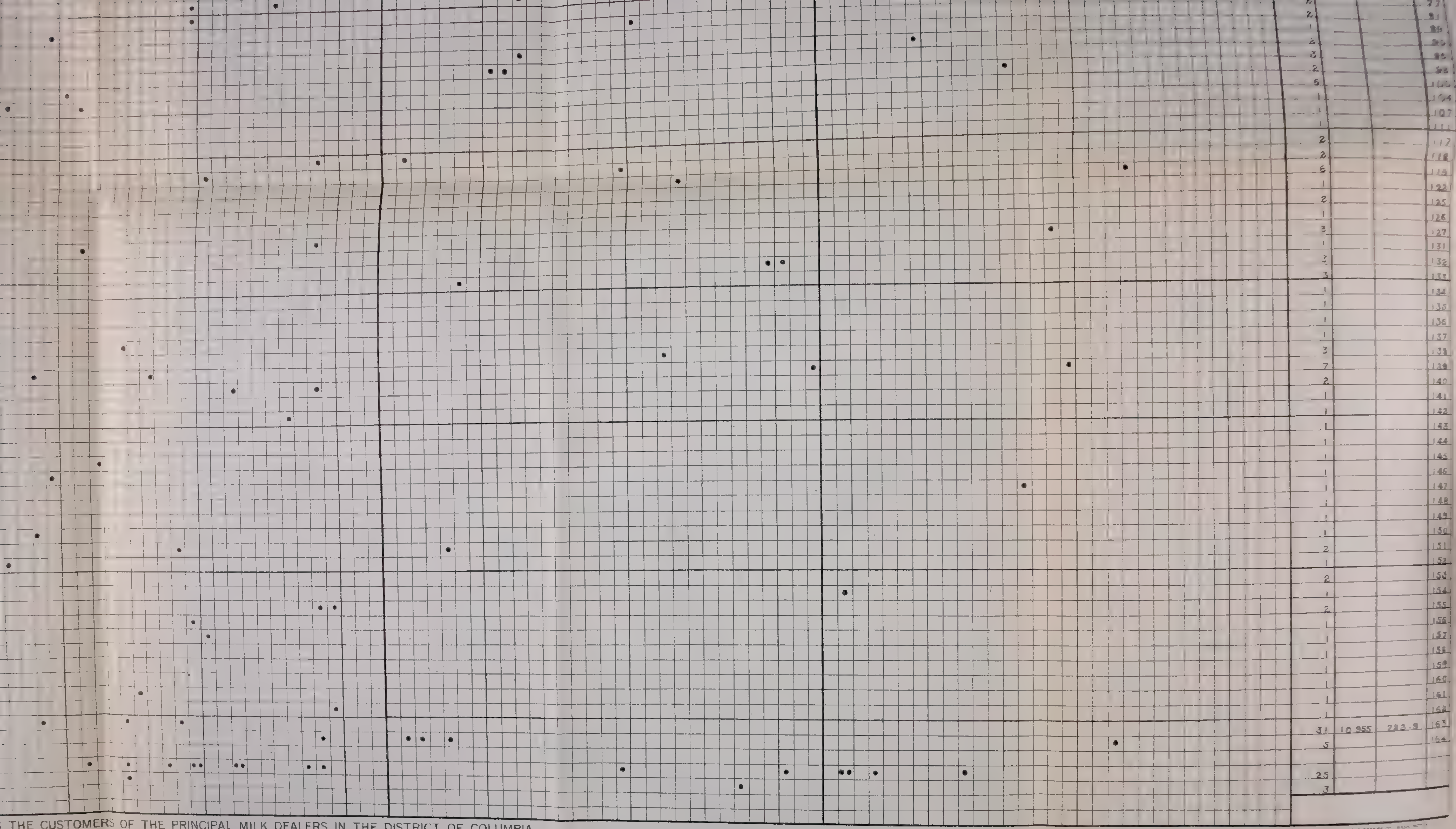
No positive evidence could be obtained that these cases were due to milk infection, yet the grouping of the cases, etc., suggest so strongly that these were small milk outbreaks that it seems fair to estimate the chances of these 40 cases as having been due to milk infection at about 50 per cent; and so, adding 20 of these cases to the 28 quite definitely attributable to milk infection, we have 48, or about 7 per



SHOWING THE NUMBER OF CASES OF TYPHOID FEVER, ACCORDING TO DATE OF ON

THE NUMBER OF THE DAIRY CORRESPONDS TO THE NUMBER OF THE DA

NOTE THE RELATIVELY SMALL NUMBER OF CASES PER 100,000 GALLONS OF MILK SOLD FO
P. H. & M. H. S. THIS DAIRY IS THE ONLY ONE IN WAS

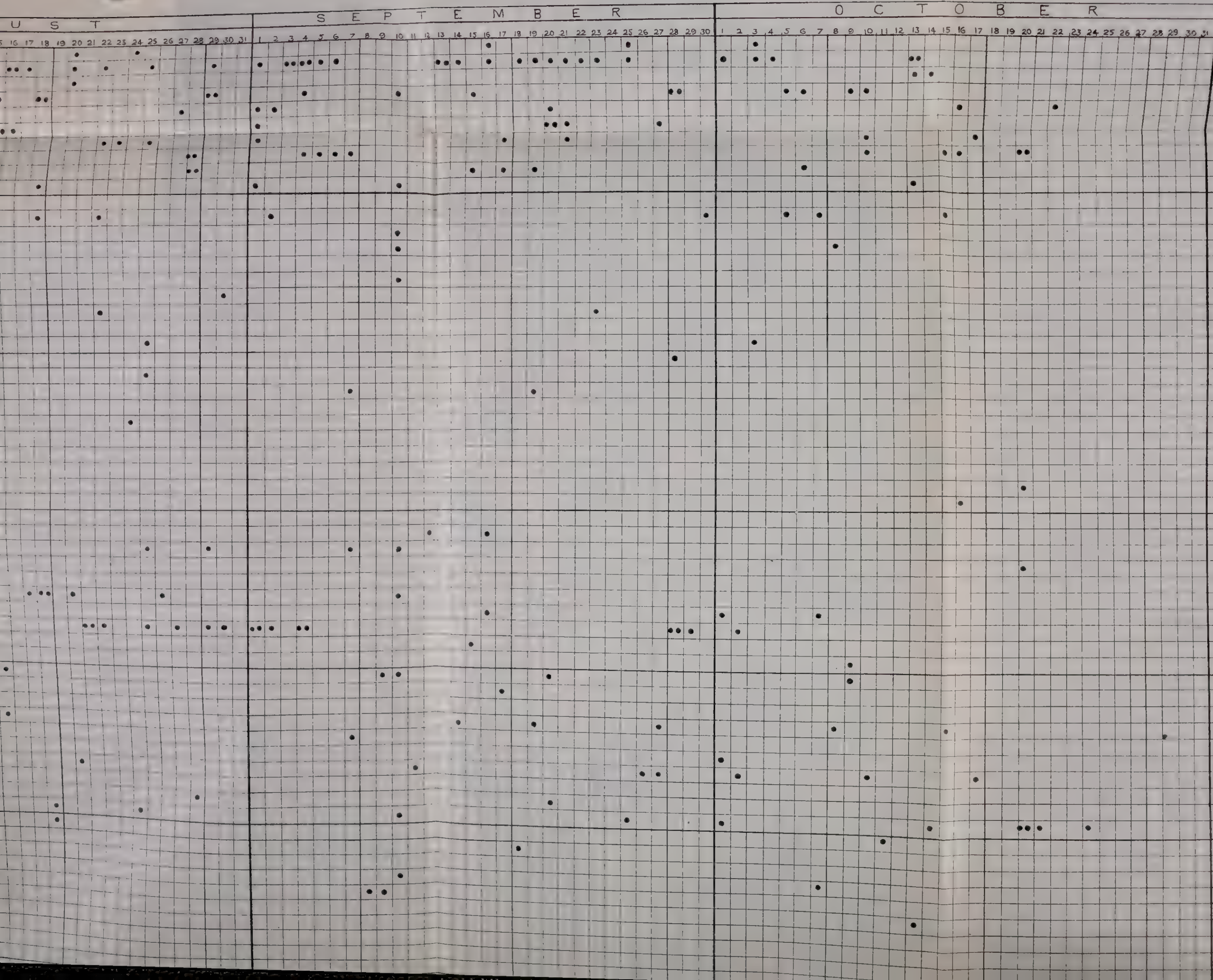


THE CUSTOMERS OF THE PRINCIPAL MILK DEALERS IN THE DISTRICT OF COLUMBIA.

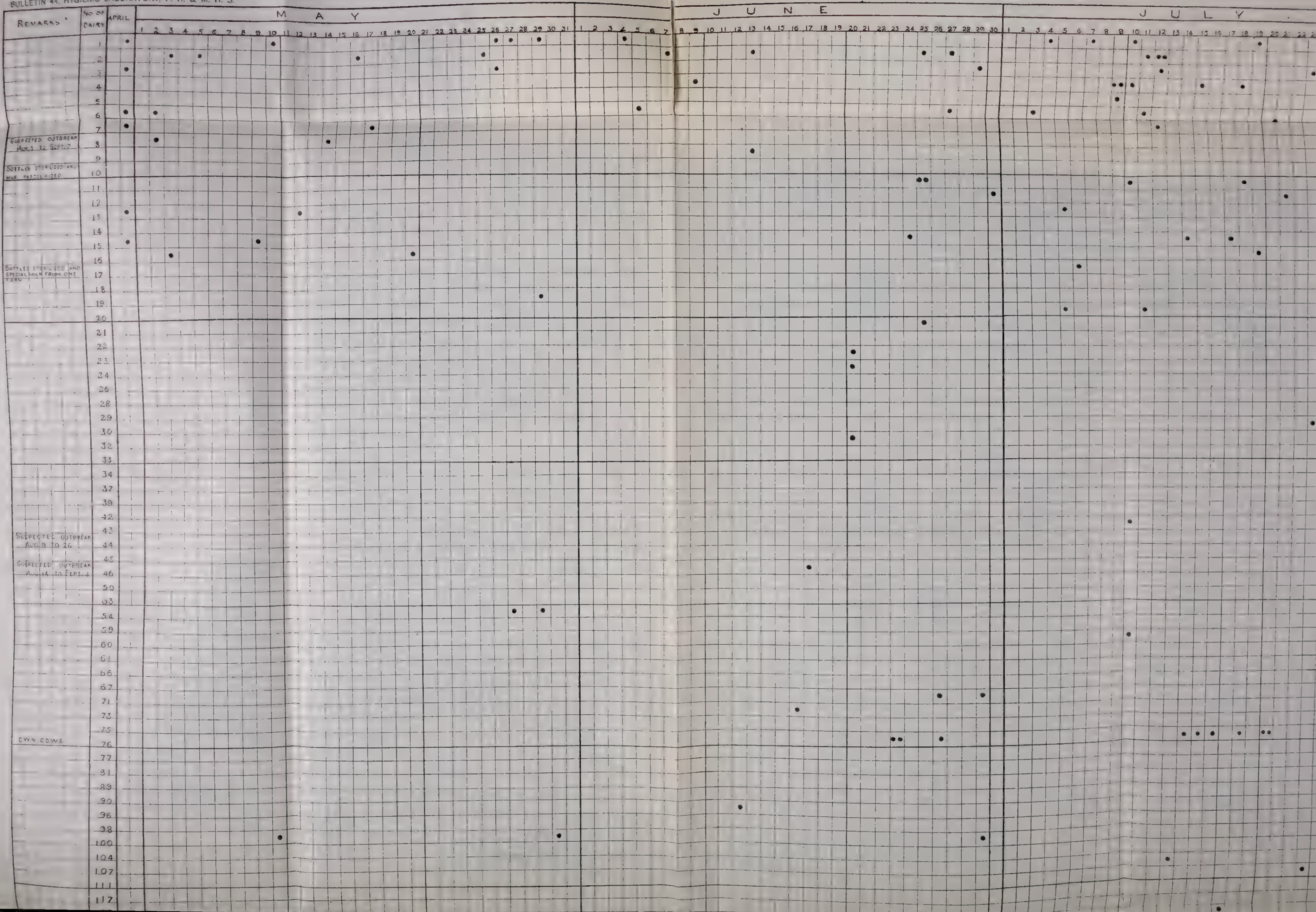
RT No. 3, BULLETIN No. 35, OF THE HYGIENIC LABORATORY, P. H. & M. H. S.

No. 10 ON THIS CHART AND ON CHART No. 3 OF BULLETIN No. 35, HYGIENIC LABORATORY,
HAT BOTH STERILIZES BOTTLES AND PASTEURIZES MILK.

CHART No. 4



NO. OF CASES	GALLONS OF MILK SOLD	NO. CASES PER 100 000 GALS MILK SOLD	NO. OF [illegible]
18	258 724	7.1	1
50	214 030	23.4	2
11	154 963	7.1	3
32	148 352	21.6	4
10	137 516	7.2	5
20	135 044	15.6	5
16	117 365	13.5	7
22	128 800	17.1	8
9	100 800	8.9	9
8	147 107	5.4	10
7	85 558	8.2	11
10	89 240	11.2	12
6	92 602	6.5	13
4			14
5			15
4	49 395	8.1	16
2	38 637	5.2	17
2	42 540	4.7	18
2			19
4			20
2			21
1			22
3			23
1			24
1			26
1			28
2			29
1			30
3			32
1			33
3			34
2			37
5			39
1			42
2			43
10	37 440	26.7	44
3			45
18	61 399	29.3	46
1			50
3			53
8			54
1			59
1			60
6			61
1			66
1			67
11			71
1			73
3			75
21			76
2			77
2			81
1			89
2			90
3			96
2			98
6			100
1			104


REMARKS
No. OF DAIRY
APRIL
M A Y
J U N E
J U L Y
SUSPECTED OUTBREAK AUG. 5 TO SEPT. 7
BOTTLES STERILIZED AND MILK PASTEURIZED
BOTTLES STERILIZED AND SPECIAL MILK FROM ONE FARM
SUSPECTED OUTBREAK AUG. 9 TO 26
SUSPECTED OUTBREAK AUG. 14 TO SEPT. 4
OWN COWS
1
2
3
4
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107
111
112

ent of the 670 cases, or 9 per cent of the 523 cases, attributable to nilk infection.

Attention is called to the difference for the two years in the ratios of number of typhoid cases to amount of milk sold of the two large nilk dealers who had outbreaks of typhoid fever among their customrs in 1906 but not in 1907 (see Chart No. 3 of Hygienic Laboratory Bulletin 35 and Chart No. 4 of this bulletin), thus:

Number of cases per 100,000 gallons of milk sold.

	1906.	1907.
ilk dealer No. 4	52.5	21.6
ilk dealer No. 13	113.9	6.5

Of the 866 cases investigated by us in 1906, about 10 per cent were ttributed to infection conveyed by milk in the course of pronounced utbreaks among the customers of three milk dealers.

Besides these cases, quite definitely attributable to infected milk, here no doubt were, during both years, scattering cases due to, but vhich could not be traced to, milk. The number of such cases is of ourse problematical.

The large proportion of cases of typhoid fever in families of the etter class and the conspicuously large proportion of cases among hildren in Washington suggest that milk plays a larger part in the pread of the infection than we have been able to definitely determine y epidemiological studies. A study of Chart No. 3 of Hygienic aboratory Bulletin 35 and the corresponding chart (No. 4) of this ulletin seems to support such a view. In this connection it is intersting to note that for both 1906 and 1907 milk dealer No. 10 had a onspicuously small number of cases of typhoid fever proportionate o the amount of milk sold. This dealer is the only one in Washingon who both sterilizes the bottles and pasteurizes the milk. The ow typhoid fever rate among the customers of milk dealer No. 10 s significant, and perhaps is a fair index of the result which would e accomplished by the pasteurization of the general milk supply of Vashington.

Considering only the cases definitely attributable to milk and the umber of secondary cases contracting the infection directly or inlirectly from them, it is evident that the milk problem in the District f Columbia is grave and important, and one requiring the serious ttention of sanitary authorities.

The milk supply of Washington is obtained from about 1,000 dairy arms scattered through Virginia, Maryland, the District of Columia, and a few even in Pennsylvania and New York. Some of this ailk is sent directly from the dairy farms to the consumer; the bulk of it, however, is shipped from the farms to the 65 or 70 dairies operating in the District of Columbia, where it is mixed, bottled, etc., before delivery. Some of the dairies in Washington receive milk from as many as 30 to 40 farms. It is evident that a supervision over these various dairy farms and dairies sufficiently thorough to prevent the introduction of typhoid fever infection into milk would require a large force of inspectors and could be done only at a great cost. Therefore the treatment of milk before it is delivered to the consumer by some process (pasteurization), which will destroy any possible infection contained therein and at the same time not impair the food value of the milk, seems to be at the present time the cheapest and most practical method of preventing the conveyance of typhoid fever infection by milk.

There can be no more objection to the pasteurization of milk for the use of adults and of children over three years old than there is to the cooking of meat. The fresh clean "certified"[a] or "inspected"[a] milk for infant feeding and special uses needs no purification. Pasteurization may not be the ideal to be attained, but until we can be assured that the general market milk is free from danger the only protection is to destroy the infection by means of heat. Pasteurization not only protects the community against much of the typhoid fever, but also against some of the tuberculosis, scarlet fever, diphtheria, infant diarrheas, etc. For a full discussion of this subject see the article on Pasteurization in Hygienic Laboratory Bulletin No. 41, page 591.

ICE CREAM.

The history of the cases in regard to the use of ice cream withi thirty days prior to the onset of illness was as follows: Three hundred and forty-five ate it from time to time, 163 ate none, and for 1 the facts were not determined. No cases this year were traced t infected ice cream, but in view of the bad sanitary conditions unde which much of the ice cream sold in Washington is made and handle (particularly by street vendors) a certain number of scattering case probably were caused by infection through ice cream.

RAW SHELLFISH.

The following table shows the history of the cases in regard to th eating of raw oysters and clams from time to time within thirty day prior to onset of illness:

Shellfish.	Yes.	No.	Not de termine
Oysters	23	488	
Clams	17	494	

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[a] For a definition of these terms see "The classification of market milk," by A. Melvin, Hygienic Laboratory Bulletin No. 41, p. 559.

The small proportion of cases giving a history of having eaten uncooked shellfish shows that shellfish this year, as was the case in 1906, could not have been the means of transmitting the infection to any considerable number of cases.

All but 9 of the 523 cases occurred between May 1 and November 1, during which period comparatively few raw oysters or clams are eaten in the District of Columbia.

OCCUPATION.

In the following table is given the occupation of the cases, along with the number of persons in the District of Columbia, according to the census of 1900, engaged in such occupations:

Occupations.	Number of cases.			Number of persons engaged in occupation, census of 1900.		
	Male.	Female.	Total.	Male.	Female.	Total.
Professional pursuits:						
Electricians	4	0	4	461	0	461
Journalists	1	0	1	398	0	398
Lawyers	5	0	5	1,445	0	1,445
Musicians and teachers of music	3	0	3	414	307	721
Officials (Government)	2	0	2	900	0	900
Physicians and surgeons	1	0	1	881	0	881
Domestic and personal service:						
Barbers, hairdressers	2	0	2	860	0	860
Bartenders	2	0	2	591	0	591
Housekeepers, stewardesses	0	1	1	0	529	529
Janitors, sextons	1	0	1	511	0	511
Laborers, not specified	17	0	17	12,476	263	12,739
Launderers, laundresses	2	1	3	474	7,192	7,666
Nurses, midwives	1	5	6	231	1,311	1,542
Restaurants, saloon keepers	3	0	3	612	112	724
Servants and waiters	2	16	18	2,898	15,231	20,129
Soldiers, sailors, marines (United States)	3	0	3	745	0	745
Watchmen, policemen, firemen, etc	2	0	2	1,667	0	1,667
Trade and transportation:						
Agents	1	0	1	1,511	0	1,511
Bookkeepers, accountants	1	2	3	837	482	1,319
Clerks, copyists	21	9	30	11,523	4,697	14,220
Draymen, hackmen, teamsters	16	0	16	3,994	0	3,994
Hostlers	5	0	5	410	0	410
Hucksters, peddlers	1	0	1	526	0	526
Merchants, dealers (retail)	2	1	3	3,945	418	4,363
Messengers, errand and office boys	5	0	5	1,345	0	1,345
Porters, helpers in stores	6	0	6	921	0	931
Salesmen, saleswomen	5	2	7	2,644	1,320	3,964
Steam railroad employees	7	1	8	1,185	(?)	1,185
Stenographers and typewriters	0	5	5	521	708	1,229
Street railway employees	7	0	7	817	0	817
Telegraph and telephone operators	2	0	2	307	(?)	307
Mechanical and manufacturing:						
Bakers	0	1	1	622	?	625
Blacksmiths	1	0	1	775	0	772
Milliners	0	1	1	0	344	344

Occupations.	Number of cases.			Number of persons engaged in occupation, census of 1900.		
	Male.	Female.	Total.	Male.	Female.	Total.
Mechanical and manufacturing—Continued.						
Boot and shoemakers, repairers	2	0	2	496	0	496
Carpenters, joiners	7	0	7	2,298	0	2,298
Dressmakers	0	2	2	0	2,993	2,993
Engineers, firemen (not locomotive)	4	0	4	1,116	0	1,116
Iron and steel workers	4	0	4	300	0	300
Manufacturers, officials, etc	1	0	1	925	0	925
Machinists	13	0	13	1,392	0	1,392
Masons, brick and stone	3	0	3	1,153	0	1,153
Paper hangers	2	0	2	363	0	363
Plasterers	1	0	1	465	0	465
Plumbers, gas and steam fitters	5	0	5	1,074	0	1,074
Printers, lithographers, pressmen	5	4	9	2,942	481	3,323
Tailors	1	0	1	616	0	616
Miscellaneous:						
Persons attending school	84	85	169	18,788	21,116	39,904
Infants and small children	25	20	45	(?)	(?)	(?)
Housewives	0	48	48	0	35,000	[a] 35,000
Postmen	2	0	2			
Inmates of institutions	3	1	4			
No occupation	1	12	13			
Tinners and stove fitters	1	0	1			
Coppersmiths	1	0	1			
Inspector, city water department	1	0	1			
Elevator boys	2	0	2			
Dyers, cleaners	1	0	1			
Prostitutes	0	1	1			
Pharmacists	1	0	1			
Chaffeurs	1	0	1			
Veterinary surgeons	1	0	1			
Manicurists	0	1	1			
Not determined	1	0	1			
Total	304	219	523			

[a] Our estimate.

It is evident that the disease was fairly uniformly distributed among persons regardless of occupation. The rate is seen to have been somewhat disproportionately high among electricians, hostlers, porters and helpers in stores, steam railroad employees, street railway employees, telegraph and telephone operators, iron and steel workers, machinists, paper hangers and plumbers.

INFECTION BY CONTACT.

Of the 523 cases, 70, or about 13 per cent, gave a history of having had within thirty days prior to onset of illness free and intimate association with typhoid patients in the febrile stage of the disease, and were attributable to infection by direct contact. In these 70 cases are not included several giving a history of contact, but considered as being more probably attributable to some other factor such as milk

The following table gives the interval in days elapsing between the definite onset of illness of the 70 secondary cases and the definite onset of the primary cases from which these 70 cases were considered to have contracted the infection:

Number of days elapsing between onset of primary and secondary case.	Number of secondary cases.	Number of days elapsing between onset of primary and secondary case.	Number of secondary cases.
7	2	35	1
8	3	36	1
9	4	39	1
10	3	40	1
11	3	41	1
12	2	44	1
14	1	46	1
16	1	48	1
17	1	51	1
18	2	53	1
19	2	54	1
20	1	Not accurately determined	6
21	1	Interval not determined, secondary cases being among physicians, nurses, helpers, or inmates of hospitals who were more or less constantly exposed	12
22	1		
24	2		
25	3		
27	1		
28	2		
29	2		
30	2	Total	70
31	1		
33	1		

These figures emphasize the importance of exercising the utmost precautions to prevent the spread of infection from typhoid fever patients from the onset to the end of the illness.

Among the cases not definitely attributable to a known factor there were 16 which gave a history of association with febrile cases suspected to be but not reported as typhoid fever. In 45 cases indirect conveyance of infection by persons or flies was strongly suggested. In determining that conveyance of infection by flies was probable the proximity of the house occupied by the primary case to that occupied by the secondary case, the number of flies[a] in the neighborhood and the ease with which they could pass from the infected excreta of the primary case to the food of the secondary case, and all other conditions thought to have any bearing on the situation, were taken into consideration.

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[a] It is difficult to estimate the part played by flies in spreading the infection of typhoid in a city having a good sewerage system. However, it is evidently advisable to lessen their number, which may be successfully done in a community by the proper disposal of manure.

Four cases gave a history of association with persons convalescing from typhoid fever.

It seems fair to estimate the chances of these 65 cases having become infected by contact at about 50 per cent; and so, adding 32 of these to the 70 considered as almost certainly having been infected by contact, there were 102, or about 19 per cent, of the 523 cases attributable to contact. These 102 cases compose about 15 per cent of the total 670 cases investigated, as compared with 6.23 per cent (estimated on the same basis of probability) of the 866 cases investigated in 1906.

In endeavoring to determine what part, if any, chronic bacillus carriers have in the dissemination of typhoid fever infection in the District of Columbia, the following data in regard to association of the 523 cases with persons who had had typhoid fever within the five years previous were obtained:

Association 30 days prior to onset with persons who had had typhoid fever within—	Amount of association.			
	Intimate.	Fairly intimate.	Slight.	Total cases.
Six months	2	1	1	4
One year	8	5	3	16
Two years	3	5	2	10
Three years	7	5	2	14
Four years	2	2	0	4
Five years	6	3	2	11
None known				464
Total	28	21	10	523

For people living in large cities, exact data as to association during a period of thirty days with persons who have had, sometime within the previous five years, an attack of typhoid fever would be almost if not quite impossible to obtain, and so the data presented in the above table refer almost entirely to association with other members of the family or with intimate friends. The number of cases giving a history of such association is, therefore, necessarily far below the actual.

Effort was made to obtain from persons who had had typhoid within five years and with whom the cases had associated specimens of feces for bacteriological examination; but this effort did not meet with much success, as many of the people showed a reluctance, from perhaps some peculiar sense of delicacy, toward collection of the specimens. Some of the specimens were too old before they reached the laboratory to give reliable results.

Data relative to newcomers in the residences at which cases occurred are given in the following table. By newcomers we mean persons who moved into a household within three months of the onset of the case.

Number of cases at private residences at which one or more newcomers lived within thirty days prior to occurrence of case	36
At hotels, hospitals, and other public institutions	21
Not determined	3
No newcomers	463
Total	523

Of the newcomers at private residences where cases occurred, 6 gave typhoid fever histories as follows: Four years prior, 1 case; five years prior, 1 case; twelve years prior, 1 case; thirteen years prior, 1 case; twenty years prior, 1 case; sixty years prior, 1 case.

For 20 cases the newcomers were said to have never had typhoid fever and for 10 cases typhoid histories of the newcomers were not obtainable.

In the endeavor to learn what part was played by servants in the transmission of typhoid fever infection to their employers, the following data were obtained:

Seventy-one, or 13 per cent, of the 523 cases occurred at homes at which servants were employed. Of the last 306 cases investigated in 1906 and of which data regarding servants were obtained, 69, or 22 per cent, occurred at homes at which servants were employed. The difference in the percentage of cases occurring at homes where there were servants for the 1906 and the 1907 periods shows very well the difference in the class of people affected during the two periods.

Of the 71 cases occurring in the 1907 period at homes at which servants were employed, 3 were at homes where white servants were employed and resided, 1 was at a home where a white servant was employed, but who went to his own home at night, 23 were at homes where colored servants were employed and resided, and 44 at homes where colored servants were employed, but who went to their own homes at night.

Of the 97 servants who were employed at the homes of these 71 cases the typhoid histories were as follows:

White resident servants who had had typhoid fever (eighteen years prior)	1
White resident servants who had not had typhoid fever	3
White nonresident servants who had had typhoid	0
White nonresident servants who had not had typhoid	1
Colored resident servants who had had typhoid (six years prior)	1
Colored resident servants who had not had typhoid	33

Colored nonresident servants who had had typhoid fever (3, two years prior; 1, four years prior; 1, five years prior; 2, six years prior; 1, ten years prior)	8
Colored nonresident servants who had not had typhoid fever	41
Colored nonresident servants of whom typhoid histories were not obtained	9

Seventeen cases occurred at hotels, hospitals, or other public institutions at which there was a shifting of the servant population; so that accurate data regarding servants could not be obtained.

In no instance was a history obtained of the existence of typhoid fever cases at the homes of the nonresident servants during the three or four weeks prior to the onset of cases, nor was there any instance in which the information obtained indicated that the infection was conveyed by the servants to the members of employers' families.

There were 30 instances in which two or more cases developed in the same house, and in these 30 houses there were, all told, 73 cases. The largest number of cases occurring in one house was 4, and there were two such instances. Of these 73 cases, 34 were attributed to infection by direct contact, 7 to milk infection, and 32 to factors not determined.

Of the cases investigated by us in 1907, there were instances in which patients were cared for during the whole or a part of their illness in buildings at which there were grocery stores, bakeries, dairies, saloons, or restaurants, as follows:

Class of establishment:	Cases.
Grocery stores	10
Bakeries	4
Dairies	5
Saloons	4
Restaurants	5

In the majority of these instances some member of the family or other person in attendance on the patient was closely associated with the business; also in the majority of instances the precautions taken in the disposal of stools, urine, etc., were decidedly inefficient.

In considering the number of ways in which infection by personal contact may occur, it is easy to understand that many cases of typhoid fever in a large city may be due to infection by contact which can not be traced.

The time seems ripe and our knowledge of the subject sufficient to warrant the regarding of typhoid fever as being in large part a contact disease, and its prophylactic treatment as a quarantinable disease.

PROPHYLAXIS.

Of the total 670 cases investigated, 310, or 46.2 per cent, were treated at hospitals. One case was treated from the time of onset of illness at a place out of the District of Columbia. The time of admission to the hospital of cases considered as having contracted the infection in the District of Columbia was as follows:

	Cases.
Within five days after onset of illness	143
Within ten days after onset of illness	57
Within fifteen days after onset of illness	12
Within twenty days after onset of illness	3
Within twenty-five days after onset of illness	3
Within thirty days after onset of illness	1
Total	219

Of the 117 cases considered as undoubtedly having contracted the infection out of the District of Columbia, 78 were treated at hospitals; and of the 30 which were considered as almost certainly having contracted the infection out of the District of Columbia, 13 were treated at hospitals. Of these 91 imported cases treated at hospitals, 67 were taken from places out of the District of Columbia directly to hospitals for treatment and 24 were taken to hospitals after being treated at private residences in the District of Columbia as follows:

	Cases.
At private residences:	
One day	7
Two days	2
Three days	2
Five days	2
Six days	1
Seven days	2
Eight days	2
Nine days	2
Ten days	1
Twelve days	2
Twenty-four days	1
Total	24

This year, as in 1906, the good percentage of cases, particularly of the imported cases, treated at hospitals was encouraging, as the facilities for preventing the spread of infection are usually much better in hospitals than at private residences.

Of the 359 cases treated at private residences, 90 were attended by professional nurses. The treatment of stools and urine with disinfectants was considered efficient for 128 cases, inefficient for 180, of doubtful efficiency for 48, and the method not ascertained for 3.

Of the 180 patients whose stools and urine were inefficiently treated no attempt at disinfection was made for 65; the treatment was inefficient on account of the small quantity of disinfectant used for 58; and on account of a shortness of time of exposure of excreta to disinfectant for 57. For 48 of the patients the treatment of the stools and urine was considered of doubtful efficiency because the agent used for accomplishing the disinfection was of doubtful value.

The large number of instances in which the stools and urine of the patients were not properly disinfected shows the need of some radical action to be taken in regard to this matter.

Of other measures to prevent the spread of the infection, such as treatment of clothing, bedding, dishes, etc., used by the patients, the precautions carried out were considered efficient for 79, fairly efficient for 96, inefficient for 181, and not determined for 3.

Of the cases investigated by us in 1906, the treatment of the stools and urine of the patients cared for at private residences was as follows:

Treatment of stools and urine:	Cases.
Efficient	145
Inefficient	286
Of doubtful efficiency	51
Method not determined	10
Total	492

Thus, of the cases investigated by us in 1906 and in 1907 for the large majority cared for at private residences, the treatment with disinfectants of the stools and urine and the other measures to prevent the spread of infection from the patients were decidedly inefficient.

SUMMARY FOR 1907.

Six hundred and seventy cases investigated May 1 to November 1, 1907:

	Cases.	Percentage.
Infection contracted out of District of Columbia	174	25.97
Infection attributed to milk	48	7.17
Infection attributed to contact	102	15.22
Accounted for	324	48.36
Unaccounted for	346	51.64
Total	670	100.00

Or, considering only the 523 cases, of which 363 undoubtedly and 160 probably contracted the infection in the District of Columbia, we have—

	Cases.	Percentage.
Infection attributed to milk	48	9.18
Infection attributed to contact	102	19.50
Accounted for	150	28.68
Unaccounted for	373	71.32
Total	523	100.00

To contrast with the above figures we reprint the following summary of our findings in 1906 from Hygienic Laboratory Bulletin No. 35, page 57:

SUMMARY FOR 1906.

Eight hundred and sixty-six cases investigated June 1 to November 1, 1906:

	Cases.	Percentage.
nfection contracted out of District of Columbia	129	14.89
nfection attributed to milk or ice cream	85	9.81
nfection attributed to contact	54	6.23
Accounted for	268	30.93
Unaccounted for	598	69.07
Total	866	100.00

Or, considering only the 747 cases, of which 681 undoubtedly and 66 probably contracted the infection in the District of Columbia, we have—

	Cases.	Percentage.
Attributed to infection by milk or ice cream	85	11.30
Attributed to infection by contact	54	7.30
Accounted for	139	18.60
Unaccounted for	608	81.40
Total	747	100.00

The above figures are necessarily not exact, but are approximations based on the findings.

CONCLUSIONS.

1. Much of the typhoid fever in the District of Columbia is imported.

2. Many cases in the District of Columbia are contracted through contact with persons, or with articles handled or soiled by persons in the febrile stage of the disease. The especial prevalence of the disease among children in the District of Columbia probably has an important bearing on the spread of the infection by contact.

3. Infected milk is one of the important known factors in the spread of the disease in the District of Columbia.

4. The filtered Potomac River water during the typhoid season of 1907 (May to September) was according to present bacteriologic standards of good sanitary quality and so far as could be ascertained was not responsible for the spread of the infection.

In the typhoid season of 1907 there were about 200 cases less than in the 1906 period. This improvement in the situation suggests that the diminution in the amount of typhoid fever in the District of Columbia was due to the improvement in the quality of the drinking water as the result of sand filtration. Positive proof of this can not now be established. However, a careful study of typhoid fever in the District of Columbia for the next three to five years will throw much light on the rôle played by the Potomac River water in previous years.

RECOMMENDATIONS.

We recommend:

1. That all cases of typhoid fever and all cases of suspected typhoid fever shall be treated as contagious and dangerous to the community. This should include isolation, placarding, the prompt disinfection of the discharges, the patient's bedding, etc.

2. That laboratory facilities be provided free of cost to aid physicians in the early diagnosis of typhoid fever and also to determine when persons who have had the disease no longer menace the public health by discharging typhoid bacilli.

3. That all milk not "certified" or "inspected"[a] shall be pasteurized, under official surveillance, especially during the typhoid fever season.

4. The enactment of a law prohibiting the handling or sale of milk or milk products in any dwelling or in any structure so situated as to render contamination of these products especially liable.

5. The enactment of a law prohibiting the care of a case of typhoid fever in any house where foods or beverages liable to convey the infection are sold or prepared for sale.

6. That in order to furnish a water supply of a satisfactory grade of purity throughout the year, additional storage reservoirs shall be constructed, or a coagulant (alum) shall be used during periods of high turbidity.

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[a] For the definition of "certified" or "inspected" milk, see "The classification of market milk," by A. D. Melvin, Hygienic Laboratory Bulletin No. 41, p. 559.

LIST OF HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH AND MARINE-HOSPITAL SERVICE.

The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, located in Washington, was authorized by act of Congress, March 3, 1901.

The following *bulletins* [Bulls. Nos. 1–7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.] have been issued:

*No. 1.—Preliminary note on the viability of the *Bacillus pestis.* By M. J. Rosenau.

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau.

*No. 3.—Sulphur dioxid as a germicidal agent. By H. D. Geddings.

No. 4.—Viability of the *Bacillus pestis.* By M. J. Rosenau.

No. 5.—An investigation of a pathogenic microbe (*B. typhi murium* Danyz) applied to the destruction of rats. By M. J. Rosenau.

*No. 6.—Disinfection against mosquitoes with formaldehyd and sulphur dioxid. By M. J. Rosenau.

No. 7.—Laboratory technique: Ring test for indol, by S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis; Microphotography with simple apparatus, by H. B. Parker.

By act of Congress approved July 1, 1902, the name of the "United States Marine Hospital Service" was changed to the "Public Health and Marine-Hospital Service of the United States," and three new divisions were added to the Hygienic Laboratory.

Since the change of name of the Service the bulletins of the Hygienic Laboratory have been continued in the same numerical order, as follows:

*No. 8.—Laboratory course in pathology and bacteriology. By M. J. Rosenau. (Revised edition March, 1904.)

*No. 9.—Presence of tetanus in commercial gelatin. By John F. Anderson.

No. 10.—Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States. By Ch. Wardell Stiles.

*No. 11.—An experimental investigation of *Trypanosoma lewisi.* By Edward Francis.

*No. 12.—The bacteriological impurities of vaccine virus; an experimental study. By M. J. Rosenau.

*No. 13.—A statistical study of the intestinal parasites of 500 white male patients at the United States Government Hospital for the Insane; by Philip E. Garrison, Brayton H. Ransom, and Earle C. Stevenson. A parasitic roundworm (*Agamomermis culicis* n. g., n. sp.) in American mosquitoes (*Culex sollicitans*); by Ch. Wardell Stiles. The type species of the cestode genus *Hymenolepis;* by Ch. Wardell Stiles.

No. 14.—Spotted fever (tick fever) of the Rocky Mountains; a new disease. By John F. Anderson.

No. 15.—Inefficiency of ferrous sulphate as an antiseptic and germicide. By Allen J. McLaughlin.

*No. 16.—The antiseptic and germicidal properties of glycerin. By M. J. Rosenau.

*No. 17.—Illustrated key to the trematode parasites of man. By Ch. Wardell Stiles.

*No. 18.—An account of the tapeworms of the genus *Hymenolepis* parasitic in man, including reports of several new cases of the dwarf tapeworm (*H. nana*) in the United States. By Brayton H. Ransom.

*No. 19.—A method for inoculating animals with precise amounts. By M. J. Rosenau.

*No. 20.—A zoological investigation into the cause, transmission, and source of Rocky Mountain "spotted fever." By Ch. Wardell Stiles.

No. 21.—The immunity unit for standardizing diphtheria antitoxin (based on Ehrlich's normal serum). Official standard prepared under the act approved July 1, 1902. By M. J. Rosenau.

*No 22.—Chloride of zinc as a deodorant antiseptic, and germicide. By T. B. McClintic.

*No. 23.—Changes in the Pharmacopœia of the United States of America. Eighth Decennial Revision. By Reid Hunt and Murray Galt Motter.

No. 24.—The International Code of Zoological Nomenclature as applied to medicine. By Ch. Wardell Stiles.

No. 25.—Illustrated key to the cestode parasites of man. By Ch. Wardell Stiles.

No. 26.—On the stability of the oxidases and their conduct toward various reagents. The conduct of phenolphthalein in the animal organism. A test for saccharin, and a simple method of distinguishing between cumarin and vanillin. The toxicity of ozone and other oxidizing agents to lipase. The influence of chemical constitution on the lipolytic hydrolysis of etheral salts. By J. H. Kastle.

No. 27.—The limitations of formaldehyde gas as a disinfectant with special reference to car sanitation. By Thomas B. McClintic.

No. 28.—A statistical study of the prevalence of intestinal worms in man. By Ch. Wardell Stiles and Philip E. Garrison.

*No. 29.—A study of the cause of sudden death following the injection of horse serum. By M. J. Rosenau and John F. Anderson.

No. 30.—I. Maternal transmission of immunity to diphtheria toxine. II. Maternal transmission of immunity to diphtheria toxine and hypersuseptibility to horse serum in the same animal. By John F. Anderson.

No. 31.—Variations in the peroxidase activity of the blood in health and disease. By Joseph H. Kastle and Harold L. Amoss.

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No. 35.—Report on the origin and prevalence of typhoid fever in the District of Columbia. By M. J. Rosenau, L. L. Lumsden, and Joseph H. Kastle. (Including articles contributed by Cr. Wardell Stiles, Joseph Goldberger, and A. M. Stimson.)

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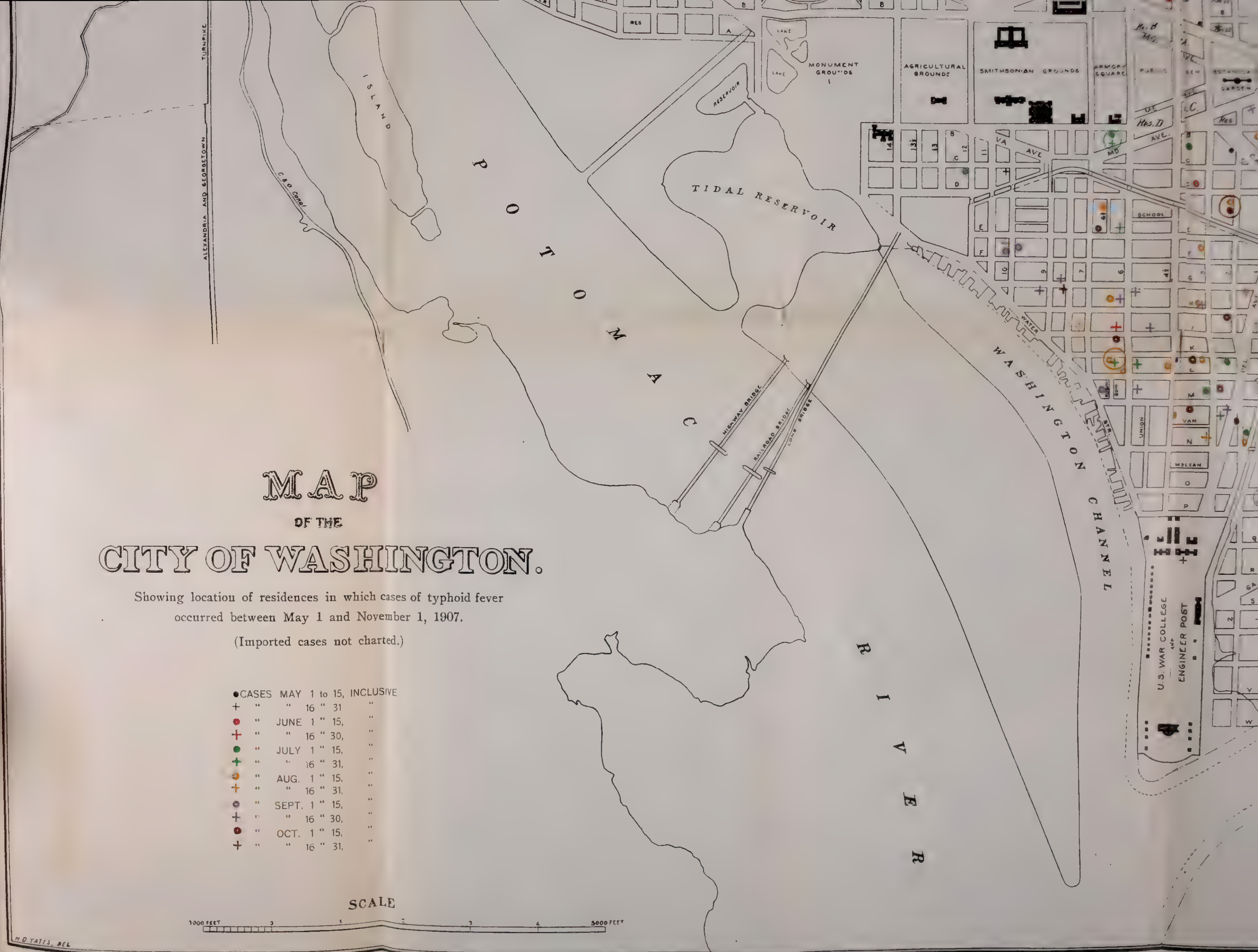

MAP OF THE CITY OF WASHINGTON.

Showing location of residences in which cases of typhoid fever occurred between May 1 and November 1, 1907.

(Imported cases not charted.)

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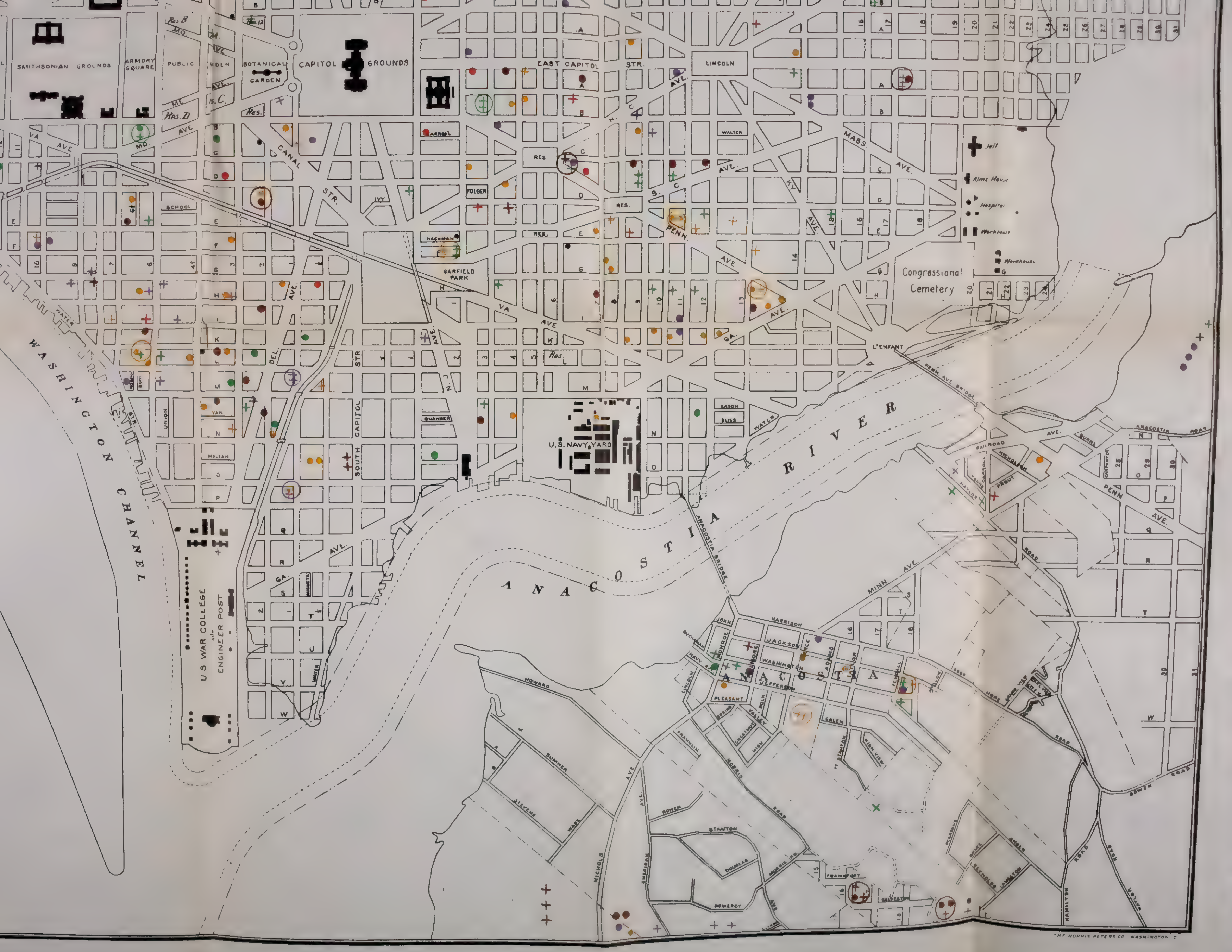

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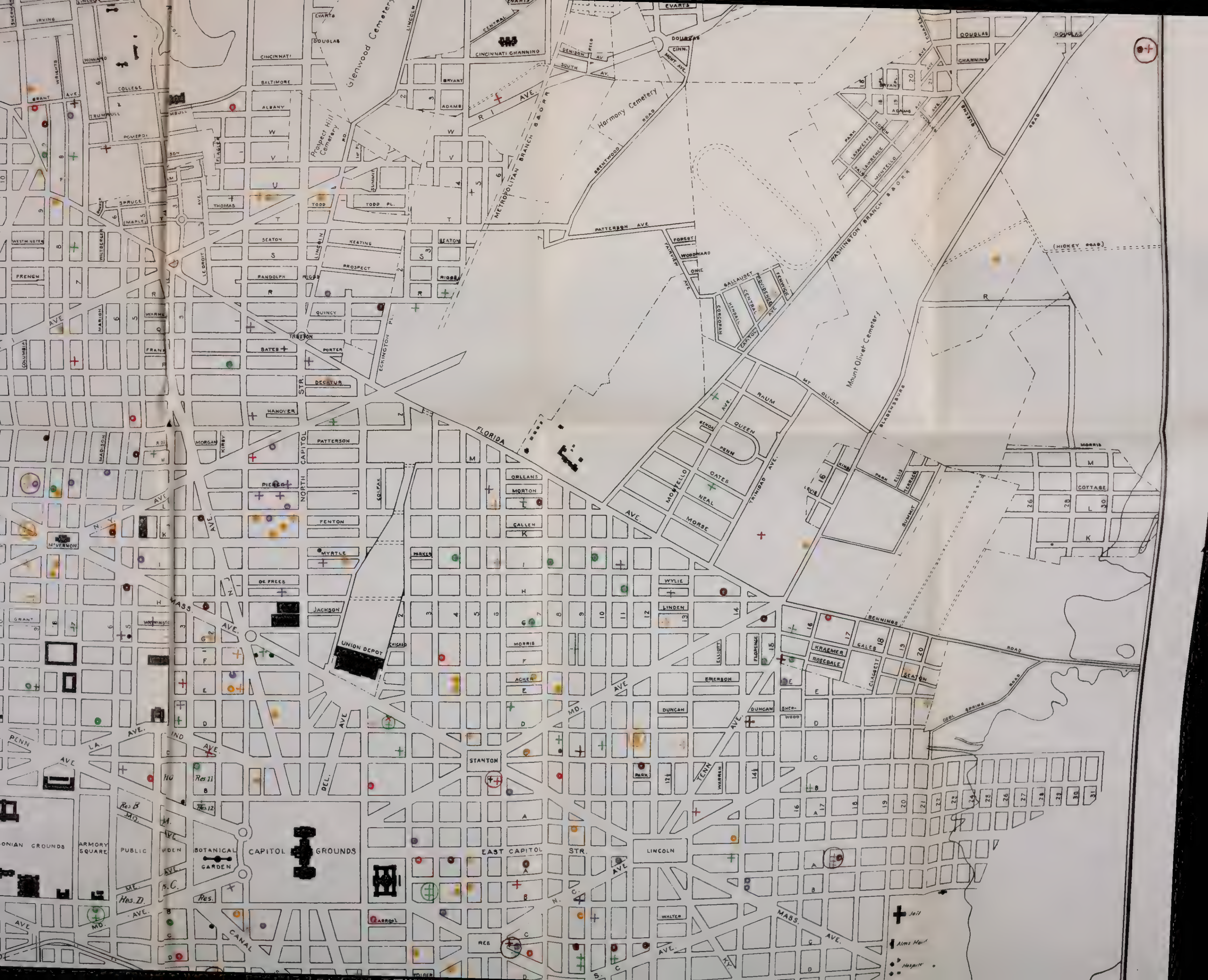
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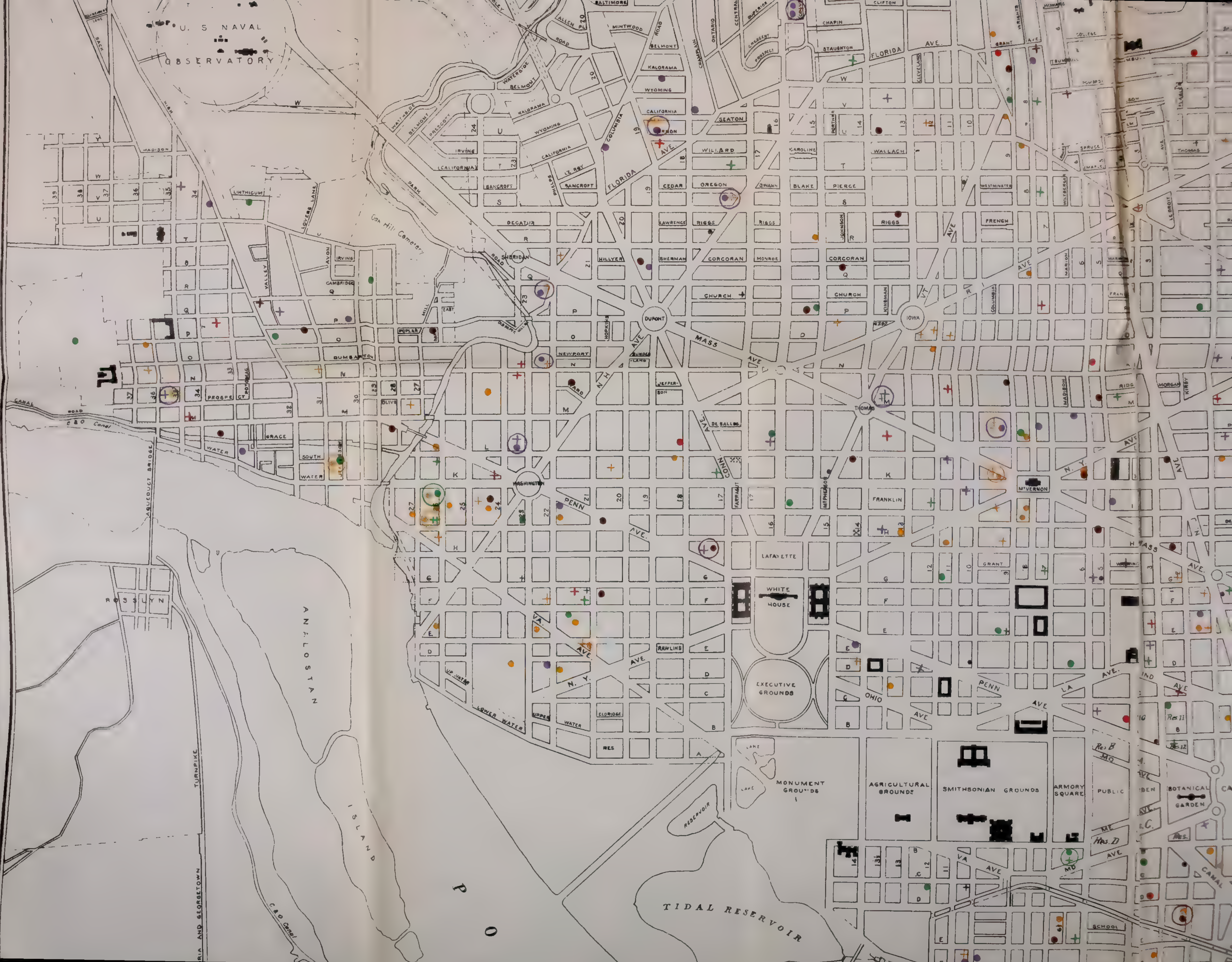
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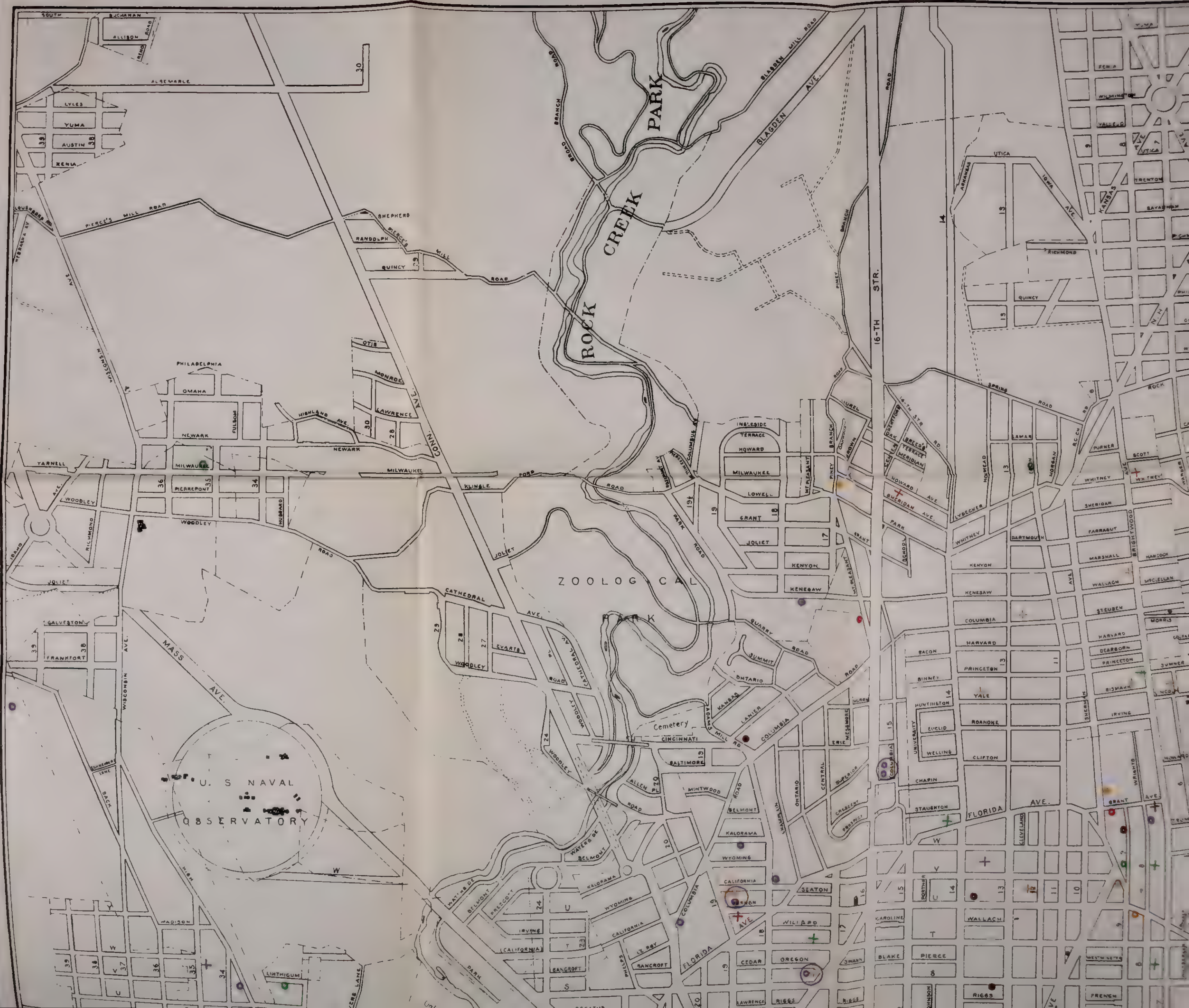
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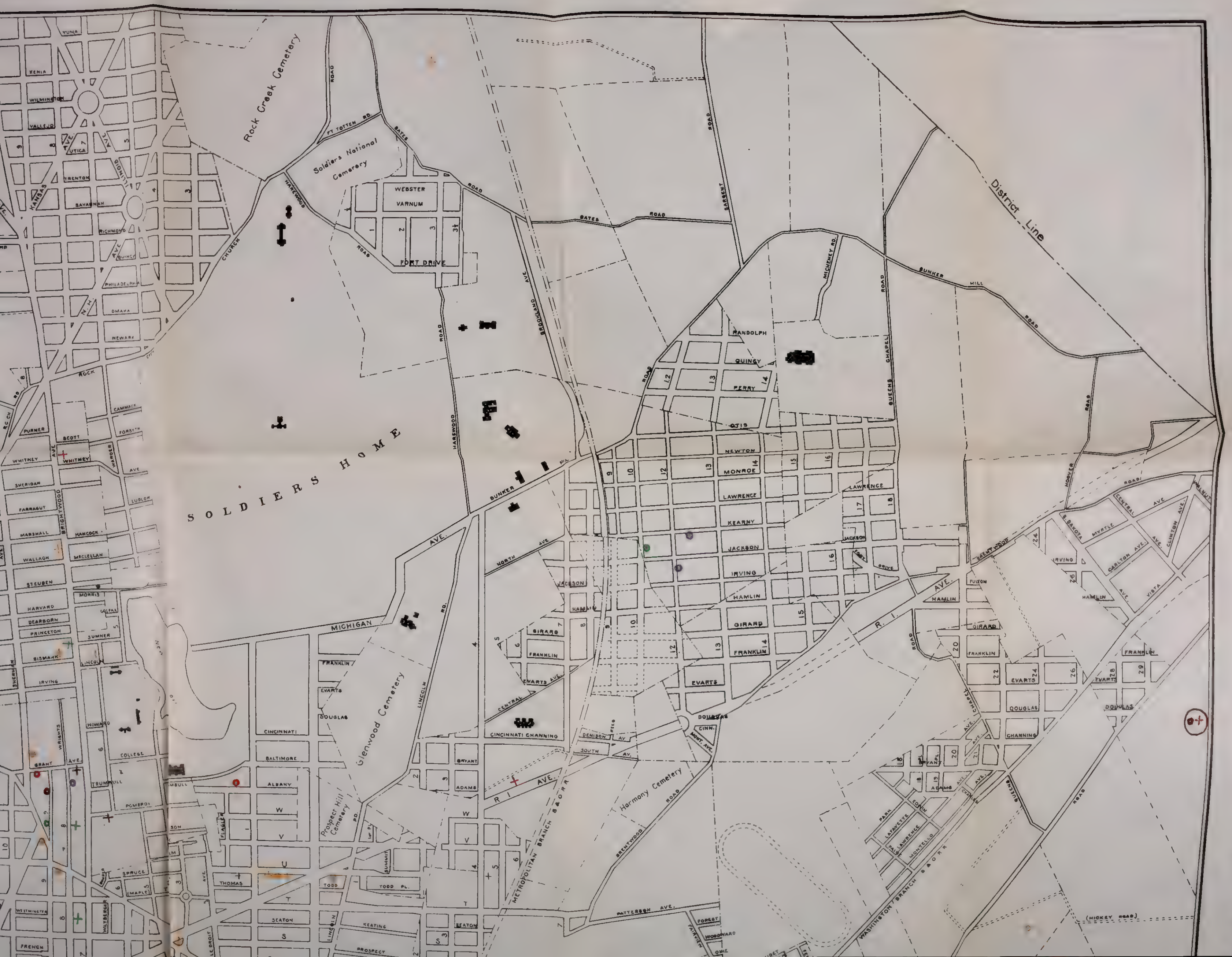
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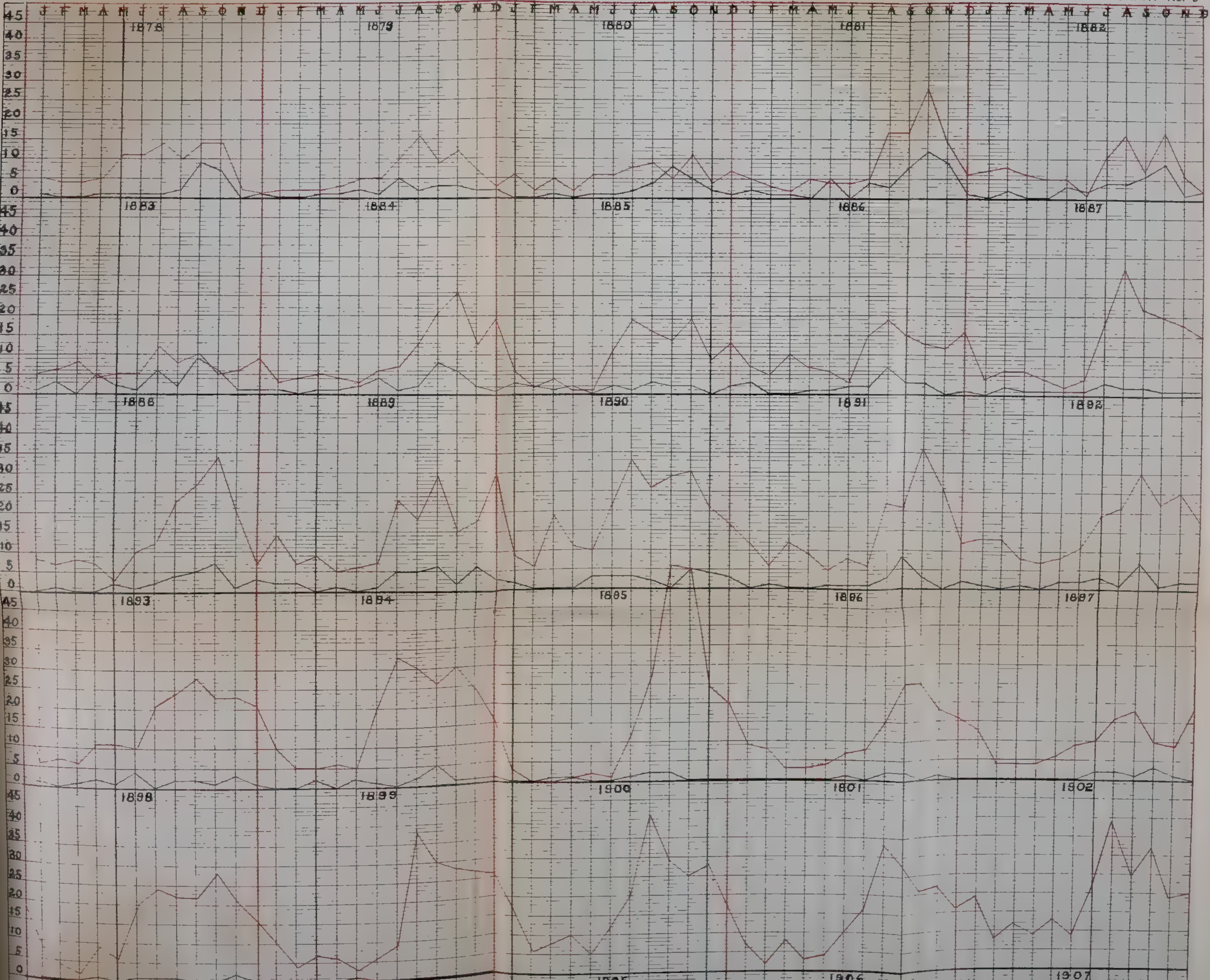
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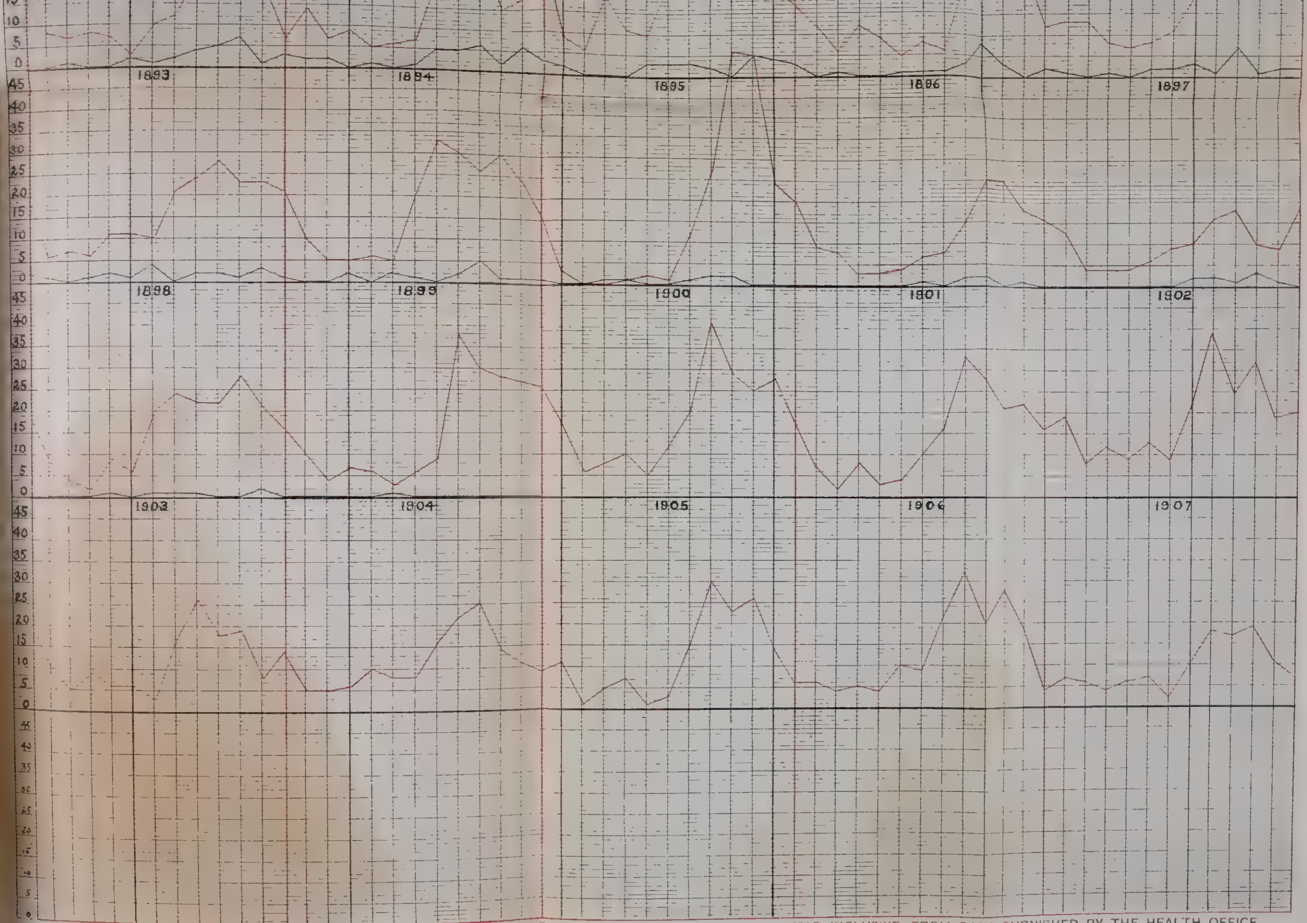

DEATHS FROM TYPHOID AND TYPHO-MALARIAL FEVERS IN THE DISTRICT OF COLUMBIA FROM 1878 TO 1907, INCLUSIVE, FROM DATA FURNISHED BY THE HEALTH OFFICE.

THE NORRIS PETERS CO., WASHINGTON, D. C.

—— RED LINES INDICATE TYPHOID FEVER.

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FURTHER STUDIES UPON ANAPHYLAXIS.[a]

By Milton J. Rosenau,
urgeon, Director Hygienic Laboratory, U. S. Public Health and Marine-Hospital Service,
and
John F. Anderson,
assed Assistant Surgeon, Assistant Director Hygienic Laboratory. U. S. Public Health and Marine-Hospital Service.

PART 1.—THE PERIOD OF INCUBATION.

In our first publication[b] from a limited series of experiments we ound the "period of incubation," or the time necessary to elapse etween the first and second injection, to be about ten days. We ere give the results of three series of tests in order to clear up he following interesting details concerning the period of incubation: 1) Does the hypersusceptibility come on abruptly or gradually? 2) Do guinea pigs sensitized in the brain have a shorter period of ncubation than those sensitized subcutaneously? (3) Is the period f incubation constant or variable?

Table No. 1.—*Period of incubation.*

. P. No.	First injection.	Interval in days.	Second injection.	Result.
436	0.01 c. c. normal horse serum subcutaneously.	4	6 c. c. normal horse serum subcutaneously.	No symptoms.
437	.....do	4	.....do	No symptoms.
438	.....do	5	.....do	No symptoms.
439	.....do	5	.....do	No symptoms.
440	.....do	6	.....do	No symptoms.
441	.....do	6	.....do	No symptoms.
442	.....do	7	.....do	No symptoms.
443	.....do	7	.....do	No symptoms.
444	.....do	8	.....do	No symptoms.
445	.....do	8	.....do	No symptoms.
446	.....do	9	.....do	Slight symptoms.
447	.....do	9	.....do	Slight symptoms.
448	.....do	10	.....do	Marked symptoms.
449	.....do	10	.....do	Marked symptoms.

---

[a] Manuscript submitted for publication May 5, 1908.

[b] Rosenau, M. J., and Anderson, John F.: A study of the cause of sudden death following the inction of horse serum. Hyg. Lab. Bul. No. 29, April, 1906, 95 p. Also Bul. No. 36, Further studies pon hypersusceptibility and immunity.

TABLE No. 2.—*Period of incubation.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1363	0.01 c. c. normal horse serum into brain.	1	6 c. c. normal horse serum intraperitoneally.	No symptoms.
1364	.....do	2	.....do	No symptoms.
1365	.....do	3	.....do	No symptoms.
1366	.....do	4	.....do	Suggestive.
1367	.....do	5	.....do	Suggestive.
1368	.....do	7	.....do	Died in 25 minutes.
1370	.....do	8	.....do	Suggestive.
1369	.....do	9	.....do	Slight symptoms.
1371	.....do	10	.....do	Dead in 35 minutes.
1372	.....do	10	6 c. c. normal horse serum subcutaneously.	Mild symptoms.

TABLE No. 3.—*Period of incubation.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1423	0.01 c. c. normal horse serum into brain.	4	6 c. c. normal horse serum subcutaneously.	No symptoms.
1424	.....do	4	.....do	No symptoms.
1425	.....do	5	.....do	No symptoms.
1426	.....do	5	.....do	No symptoms.
1428	.....do	6	.....do	No symptoms.
1429	.....do	6	.....do	No symptoms.
1427	.....do	7	.....do	No symptoms.
1430	.....do	7	.....do	Slight symptoms.
1431	.....do	8	.....do	Slight symptoms.
1432	.....do	8	.....do	Slight symptoms.
1433	.....do	9	.....do	Slight symptoms.
1434	.....do	9	.....do	Slight symptoms.
1435	.....do	10	.....do	Marked symptoms.

It will be seen from the above work that the period of incubation is about seven days or less in guinea pigs sensitized in the brain and about nine days in guinea pigs sensitized subcutaneously. So far as may be judged, it therefore appears that the period of incubation is somewhat shorter in guinea pigs sensitized in the brain than those sensitized subcutaneously. It also seems quite evident that the sensitization comes on somewhat gradually. Judged by our own results and the work of others, the period of incubation is quite constant.

Lewis[a] states that the incubation period is not to be considered as abruptly terminating at a given day. He says that he has made an animal quite sick by the intracardial injection of 2 c. c. of serum on the sixth day after a toxine-antitoxin mixture.

---

[a] Lewis, Paul A.: The induced susceptibility of the guinea pig to the toxic action of the blood serum of the horse. Journ. Exper. Med., vol. 10, No. 1, Jan. 1, 1908, p. 1.

RELATION OF THE AMOUNT OF THE SENSITIZING DOSE TO THE TIME INTERVAL.

It seemed to us at one time in our work that animals injected with a large quantity of horse serum at the first injection had a more prolonged period of incubation or were less sensitive than guinea pigs given the usual minute amounts. We conjectured that this might have been due to the slow absorption of the serum, thus having somewhat the immunizing effect of repeated injections. We therefore prepared four series of guinea pigs: One sensitized with 0.01 c. c., a second with 0.1 c.c., a third with 1 c. c., and a fourth with 8 c. c. of normal horse serum. All of these animals were subsequently tested by intracerebral injections of the same normal horse serum.

So far as may be judged from this work, the period of incubation is not appreciably prolonged by a large sensitizing dose. Further, the animals sensitized with horse serum alone, remain so for a long period of time (245 days).

TABLE NO. 4.—*Relation of amount to sensitization.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1148	0.01 c. c. normal horse (roan) serum subcutaneously.	14	0.25 c. c. normal horse (roan) serum into brain.	Marked symptoms.
1149	.....do	17	.....do	Dead in 5 minutes.
1150	.....do	24	.....do	Severe symptoms.
1151	.....do	38	.....do	Severe symptoms.
1152	.....do	110	.....do	Dead in 6 minutes.
1155	.....do	155	.....do	Very severe symptoms.
1157	.....do	245	0.2 c. c. normal horse (roan) serum into brain.	Mild symptoms.
1158	0.1 c. c. normal horse (roan) serum subcutaneously.	14	0.25 c. c. normal horse (roan) serum into brain.	Very severe symptoms.
1159	.....do	17	.....do	Severe symptoms.
1160	.....do	24	.....do	Severe symptoms.
1161	.....do	38	.....do	Dead in 4 minutes.
1163	.....do	110	0.5 c. c. normal horse (roan) serum into brain.	Dead in 4 minutes.
1164	.....do	155	0.25 c. c. normal horse (roan) serum into brain.	Very severe symptoms.
1165	1 c. c. normal horse (roan) serum subcutaneously.	14	0.25 c. c. normal horse (roan) serum into brain.	Very severe symptoms.
1166	.....do	17	.....do	Severe symptoms.
1167	.....do	24	.....do	Severe symptoms.
1168	.....do	24	.....do	Severe symptoms.
1169	.....do	110	.....do	Very severe symptoms.
1170	.....do	155	.....do	Very severe symptoms.
1162	.....do	245	0.2 c. c. normal horse (roan) serum into brain.	Mild symptoms.

TABLE No. 4.—*Relation of amount to sensitization*—Continued.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1172	8 c. c. normal horse (roan) serum subcutaneously.	14	0.25 c. c. normal horse (roan) serum into brain.	Severe symptoms.
1173	.....do	17	.....do	Severe symptoms.
1174	.....do	24	.....do	Severe symptoms.
1175	.....do	38	.....do	Severe symptoms.
1177	.....do	110	0.5 c. c. normal horse (roan) serum into brain.	Very severe symptoms.
1176	.....do	155	0.25 c. c. normal horse (roan) serum into brain.	Very severe symptoms.
1178	.....do	245	0.2 c. c. normal horse (roan) serum into brain.	Mild symptoms.

PART 2.—THE SENSITIZING PRINCIPLE.

THE EFFECT OF HEAT UPON THE SENSITIZING ACTION OF HORSE SERUM.

In previous publications we reported that the toxic action of horse serum was not destroyed when the serum was heated to 60° C. for six hours, but is entirely destroyed at 100° C. in fifteen minutes. Further work on this subject leads us to conclude that the sensitizing effect of horse serum is gradually influenced by heat, and almost entirely disappears when the serum is heated to 100° C. for one hour. The pigs sensitized with small quantities of horse serum heated to 100° C. for one hour, when subsequently tested, developed very slight symptoms.

Table No. 5.—*Effect of heat upon sensitizing principle.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
	Heated to 60° C.			
1193	0.02 c. c. normal horse (roan) serum, diluted 1:3 with distilled water and heated 60° for 1 hour, subcutaneously.	25	0.25 c. c. normal horse (roan) serum into brain.	Very severe symptoms.
	Heated to 70° C.			
1194	0.02 c. c. normal horse (roan) serum, diluted 1:3 with distilled water and heated 70° for 1 hour, subcutaneously.	25	6 c. c. normal horse (roan) serum intraperitoneally.	Slight symptoms.
1195	.....do........................	25	0.25 c. c. normal horse (roan) serum into brain.	Dead in 4 minutes.
	Heated to 80° C.			
1197	0.02 c. c. normal horse (roan) serum, diluted 1:3 with distilled water and heated 80° for 1 hour, subcutaneously.	25	6 c. c. normal horse (roan) serum intraperitoneally.	Marked symptoms; died 2 days later.
1196	.....do........................	25	0.25 c. c. normal horse (roan) serum into brain.	Severe symptoms.
3242	.....do........................	39	6 c. c. normal horse (roan) serum intraperitoneally.	Mild symptoms.
3244	.....do........................	39	.....do........................	Severe symptoms.
3240	.....do........................	39	.....do........................	Severe symptoms.
	Heated to 90° C.			
1199	0.02 c. c. normal horse (roan) serum, diluted 1:3 with distilled water and heated 90° for 1 hour, subcutaneously.	25	.....do........................	No symptoms.
1198	.....do........................	25	0.25 c. c. normal horse (roan) serum into brain.	No symptoms.
3238	.....do........................	39	6 c. c. normal horse (roan) serum intraperitoneally.	Dead in 90 minutes.
3235	.....do........................	39	.....do........................	No symptoms.
3239	.....do........................	39	.....do........................	Marked symptoms.

TABLE No. 5.—*Effect of heat upon sensitizing principle*—Continued.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
	Heated to 100° C.			
1200	0.02 c. c. normal horse (roan) serum, diluted 1:3 with distilled water and heated 100° for 1 hour, subcutaneously.	25	6 c. c. normal horse (roan) serum intraperitoneally.	No symptoms.
1201	.....do...........................	25	0.25 c. c. normal horse (roan) serum into brain.	No symptoms.
1358	1 c. c. normal horse (roan) serum, diluted 1:3 with distilled water and heated 100° for 1 hour, subcutaneously.	35	0.2 c. c. normal horse (roan) serum into brain.	No symptoms.
1359	.....do...........................	35	.....do...........................	Symptoms (?).
1360	.....do...........................	35	6 c. c. normal horse (roan) serum intraperitoneally.	No symptoms.
1361	.....do...........................	35	6 c. c. normal horse (roan) serum subcutaneously.	Symptoms (?).
1362	.....do...........................	35	.....do...........................	Symptoms (?).

CAN GUINEA PIGS BE SENSITIZED IN THE BRAIN?

We were led to make the following experiments in view of a statement made by Besredka and Steinhardt [a] that guinea pigs were not sensitized when very small amounts are injected into the brain. Very minute amounts when injected into the brain in our experiments, as well as the few reported by Besredka and Steinhardt, proved negative.

However, guinea pigs may be readily sensitized by intracerebral injections, provided quantities of 0.0001 c. c. or more are used. We obtained negative results with sensitizing doses of 0.00001 c. c.

TABLE No. 6.—*Intracerebral sensitization.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1202	0.25 c. c. normal horse (roan) serum into brain.	25	6 c. c. normal horse (roan) serum intraperitoneally.	Severe symptoms.
1203	.....do...........................	25	0.25 c. c. same serum into brain..	Marked symptoms.
1204	0.1 c. c. normal horse (roan) serum into brain.	25	6 c. c. same serum intraperitoneally.	Mild symptoms.
1205	.....do...........................	25	0.25 c. c. same serum into brain..	Marked symptoms.
1207	0.01 c.c. normal horse (roan) serum into brain.	25	6 c. c. same serum intraperitoneally.	Severe symptoms.
1206	.....do...........................	25	0.25 c. c. same serum into brain..	Marked symptoms
1208	0.001 c. c. normal horse (roan) serum into brain.	25	6 c. c. same serum intraperitoneally.	Severe symptoms.
1209	.....do...........................	25	0.25 c. c. same serum into brain..	Slight symptoms.
1210	0.0001 c. c. normal horse (roan) serum into brain.	25	6 c. c. same serum intraperitoneally.	Marked symptoms
1211	.....do...........................	25	0.25 c. c. same serum into brain..	Slight symptoms.
1213	0.00001 c.c. normal horse (roan) serum into brain.	25	6 c. c. same serum intraperitoneally.	No symptoms.
1212	.....do...........................	25	0.25 c. c. same serum into brain..	No symptoms.

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[a] Ann. de l'Inst. Pasteur, vol. 21, 1907, p. 384.

GUINEA PIGS MAY BE SENSITIZED IN THE EYE.

So far as may be judged from the following experiment, it appears that normal horse serum dropped upon the eye of a guinea pig may sensitize it to a second injection of horse serum given after an interval of seventeen days.

Table No. 7.—*Ocular sensitization.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1340	1 drop normal horse serum into each eye.	17	6 c. c. normal horse (roan) serum intraperitoneally.	Mild symptoms.
1341	.....do	28	6 c. c. normal horse (roan) serum subcutaneously.	No symptoms.
1342	.....do	28	.....do	No symptoms.

ATTEMPTS TO SENSITIZE GUINEA PIGS WITH PURE PROTEINS.

We have assumed that it is a protein substance in the horse serum, egg-white, milk, vegetable extracts, etc., that we have tested which sensitizes guinea pigs and poisons them at the second injection. The substances used by us of course have a very complex composition, so that it became desirable to use solutions of pure proteins for these tests. We are indebted to Dr. Lafayette B. Mendel, New Haven, Conn., for a quantity of edestin and excelsin, which are protein substances obtained in a chemically pure crystalline state. Our tests with these two particular proteins resulted negatively, due probably to the difficulty of obtaining a satisfactory solution of them.

Table No. 8.—*Pure protein.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1393	0.1 gm. edestin subcutaneously...	21	3 c. c. 1 per cent solution edestin in 10 per cent NaCl subcutaneously.	No symptoms.
1394	0.01 gm. edestin subcutaneously..	21	.....do	No symptoms.
1395	0.001 gm. edestin subcutaneously.	21	.....do	No symptoms.
1396	0.1 gm. edestin subcutaneously...	22	6 c. c. 1 per cent solution edestin in 10 per cent NaCl subcutaneously.	No symptoms.
1397	0.01 gm. edestin subcutaneously..	22	.....do	No symptoms.
1398	0.001 gm. edestin subcutaneously.	22	.....do	No symptoms.
1399	0.1 gm. excelsin subcutaneously...	21	3 c. c. 1 per cent solution excelsin in 10 per cent NaCl subcutaneously.	Symptoms(?).
1400	0.01 gm. excelsin subcutaneously..	21	.....do	Symptoms(?).
1401	0.001 gm. excelsin subcutaneously	21	4 c. c. 1 per cent solution excelsin in 10 per cent NaCl subcutaneously.	Symptoms(?).
1402	0.1 gm. excelsin subcutaneously...	22	6 c. c. 1 per cent solution excelsin in 10 per cent NaCl subcutaneously.	No symptoms.
1403	0.01 gm. excelsin subcutaneously..	22	.....do	No symptoms.

THE EFFECT OF BLOOD SERUM AND BRAIN OF SENSITIZED GUINEA PIGS UPON THE SENSITIZING SUBSTANCES.

Normal horse serum was mixed with an equal volume of the blood serum of a sensitive guinea pig and allowed to stand twenty-four hours at room temperature before the mixture was injected into a normal guinea pig.

Normal horse serum was also mixed with the brain substance of a sensitive guinea pig, well rubbed up in a mortar, and allowed to stand at room temperature twenty-four hours. The extract, strained through gauze, was injected subcutaneously into normal guinea pigs.

After a proper period of incubation these animals were tested and it was found that the anaphylactic phenomenon was in no wise modified by these procedures.

TABLE NO. 9.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1406	1 c. c. mixture of serum of G. P. 9558 +normal horse (roan) serum, equal parts, subcutaneously.	19	0.2 c. c. normal horse (Teddy) serum into brain.	Severe symptoms.
1407	.....do.............................	19	.....do......................	Severe symptoms.
1405	1 c. c. mixture of brain of G. P. 9558+normal horse (roan) serum, equal parts, subcutaneously.	19	.....do......................	Severe symptoms.
1408	.....do.............................	19	.....do......................	Severe symptoms.

Guinea pig No. 9558 received a subcutaneous injection of .25 c. c. of diphtheria toxine No. 7 + 1/340 c. c. antitoxic serum (Welc. 839) 61 days before being bled.

PART 3.—THE TOXIC PRINCIPLE.

THE EFFECT OF HEAT UPON THE TOXICITY OF HORSE SERUM.

It will be seen from the following tables that normal horse serum may be heated to 90° C. for one hour and still remain slightly toxic when injected into a sensitized guinea pig. Its toxicity, however, is evidently markedly affected. Heating to 70° C. for one hour does not seem to appreciably diminish its poisonous properties, but it appears to be affected at 80° for one hour. At 100° for one hour the toxicity apparently disappears.

Blood serum of course can not be heated to these high degrees without coagulation, and it is therefore necessary to dilute it in the proportion of one part of blood serum to three parts of distilled water. This dilution may then be heated to a high temperature without producing any visible change other than a slight opalescence.

It appears that there is a slight difference between the sensitizing and toxic principles in horse serum so far as resistance to heat is concerned. Serum heated to 100° C. for one hour retains some power of sensitization, but seems to lose its toxicity when given at the second injection. This difference may be more apparent than real, for exceedingly minute amounts are sufficient to sensitize guinea pigs, while a very large quantity of weakened serum would be necessary to produce symptoms. It must be remembered that in our experiments 20 c. c. of the dilution represents but 5 c. c. of the serum.

These facts must be considered in drawing conclusions from work upon split proteins, fractional precipitation, or other methods to isolate the sensitizing substance in pure form. A very minute amount of the original protein substance in horse serum clinging to the globulins or other substances modified by chemical methods might be sufficient to sensitize guinea pigs, whereas it would require very large amounts of such a modified protein to poison a sensitive animal.

TABLE No. 10.—*The effect of heat upon the toxic principle.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
			Heated to 70° C. 1 hour.	
531	0.0006 c. c. tetanus toxine A+0.22 c. c. antitoxic horse serum (Ehrl.).	67	0.25 c. c. normal horse (roan) serum diluted 1:3, heated, concentrated, then injected into brain.	Marked symptoms.
533	0.0006 c. c. tetanus toxine A+0.24 c. c. antitoxic horse serum (Ehrl.).	67	.....do..........................	Marked symptoms.
8496	0.24 c. c. toxine No. 42+1/500 c. c. antitoxic horse serum (Cutter 1858).	69	10 c. c. same dilution (=2.5 c. c. serum) intraperitoneally.	Dead in 40 minutes.
8495	.....do..........................	69	.....do..........................	Dead in 32 minutes.
			Heated to 80° C. 1 hour.	
8380	0.24 c. c. toxine No. 42+1/240 c. c. antitoxic horse serum (PD 08043).	54	10 c. c. same dilution (=2.5 c. c. serum) intraperitoneally.	Very severe symptoms.
8494	0.24 c. c. toxine No. 42+1/300 c. c. antitoxic horse serum (Cutter 1858).	42	.....do..........................	Slight symptoms.
8857	0.245 c. c. toxine No. 42+1/400 c. c. antitoxic horse serum (Hubbert).	67	.....do..........................	Slight symptoms.
8886	0.245 c. c. toxine No. 42+1/150 c. c. antitoxic horse serum (Alex. A245).	67	.....do..........................	Severe symptoms.
8890	0.245 c. c. toxine No. 42+1/200 c. c. antitoxic horse serum (Natl. VI, 15).	67	.....do..........................	No symptons.
8883	0.245 c. c. toxine No. 42+1/440 c. c. antitoxic horse serum (Alex. A262).	67	.....do..........................	No symptoms.
8872	0.245 c. c. toxine No. 42+1/660 c. c. antitoxic horse serum (Ldrl. 19C).	67	.....do..........................	No symptoms.
			Heated to 90° C. 1 hour.	
8869	0.245 c. c. toxine No. 42+1/240 c. c. antitoxic horse serum (Str. 61S).	67	10 c. c. same dilution (=2.5 c. c. serum) intraperitoneally.	No symptoms.
8880	0.245 c. c. toxine No. 42+1/400 c. c. antitoxic horse serum (Mul. 2321).	67	.....do..........................	Severe symptoms.
8878	0.245 c. c. toxine No. 42+1/200 c. c. antitoxic horse serum (Mul. 2350).	67	.....do..........................	Mild symptoms.
8889	0.245 c. c. toxine No. 42+1/250 c. c. antitoxic horse serum (Natl. VI, 15).	67	.....do..........................	Slight symptoms.
8873	0.245 c. c. toxine No. 42+1/800 c. c. antitoxic horse serum (Ldrl. 19C).	67	.....do..........................	Slight symptoms.

TABLE No. 10.—*The effect of heat upon the toxic principle*—Continued.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
			Heated to 100° C. 1 hour.	
9260	0.23 c. c. toxine No. 7+1/700 c. c. antitoxic horse serum (Ldrl. 38B).	42	10 c. c. same dilution (=2.5 c. c. serum) intraperitoneally.	No symptoms.
9218	0.23 c. c. toxine No. 7+1/1100 c. c. antitoxic horse serum (Ldrl. 21C).	42	10 c. c. same dilution (=2.5 c. c. serum) subcutaneously.	No symptoms.
9215	0.23 c. c. toxine No. 7+1/1080 c. c. antitoxic horse serum (Ldrl. 21).	42	0.25 c. c. same dilution (=2.5 c. c. serum) into brain.	No symptoms.
9210	0.23 c. c. toxine No. 7+1/700 c. c. antitoxic horse serum (Ldrl. 19B).	42	.....do............................	No symptoms.
9343	0.23 c. c. toxine No. 7+1/600 c. c. antitoxic horse serum (Ldrl. 16F).	96	20 c. c. same dilution (=2.5 c. c. serum) intraperitoneally. Next day 6 c. c. unheated normal horse (Frank) serum intraperitoneally.	No symptoms. Severe symptoms.
9344	0.23 c. c. toxine No. 7+1/700 c. c. antitoxic horse serum (Ldrl. 16F).	96	20 c. c. dilution heated.......... Next day 6 c. c. unheated (Frank) serum.	No symptoms. Severe symptoms.

DOES HORSE SERUM DECREASE IN TOXICITY WITH AGE?

Besredka[a] states that freshly drawn horse serum is more toxic than the same serum thirty days old. Our own results are not in harmony with this conclusion, as may be seen from the following series.

TABLE No. 11.—*Effect of age upon toxicity of horse serum.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
			Serum 2 days old.	
8895	0.245 c. c. toxine No. 42+1/180 c. c. antitoxic horse serum (Cutter) subcutaneously.	67	6 c. c. normal horse (roan) serum subcutaneously.	Dead in 60 minutes.
8897	0.254 c. c. toxine No. 42+1/250 c. c. antitoxic horse serum (Cutter).	67	.....do............................	Dead in 14 hours.
8871	0.245 c. c. toxine No. 42+1/500 c. c. antitoxic horse serum (Ldrl. 19C).	67	.....do............................	Dead in 2 hours.
8896	0.245 c. c. toxine No. 42+1/200 c. c. antitoxic horse serum (Cutter).	67	.....do............................	Very severe symptoms.
8858	0.245 c. c. toxine No. 42+1/300 c. c. antitoxic horse serum (Hubbert).	67	.....do............................	Dead in 50 minutes.

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[a] Ann. de l'Inst. Pasteur, vol. 21, 1907, p. 777.

TABLE No. 11.—*Effect of age upon toxicity of horse serum*—Continued.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
			Same serum 15 days old.	
8856	0.245 c. c. toxine No. 42+1/330 c. c. antitoxic horse serum (Hubbert)	112	6 c. c. normal horse (roan) serum subcutaneously.	Dead in 40 minutes.
8859	0.245 c. c. toxine No. 42+1/400 c. c. antitoxic horse serum (Hubbert).	112	.....do.........................	Dead in 40 minutes.
8860	0.245 c. c. toxine No. 42+1/330 c. c. antitoxic horse serum (Hubbert).	112	.....do.........................	Very severe symptoms.
			Same serum 40 days old.	
9221	0.123 c. c. toxine No. 7+1/420 c. c. antitoxic horse serum (Alex. A283).	40	6 c. c. normal horse (roan) serum intraperitoneally.	Dead in 25 minutes.
9200	0.123 c. c. toxine No. 7+1/560 c. c. antitoxic horse serum (Ldrl. 19).	40	.....do.........................	Dead in 27 minutes.
9257	0.123 c. c. toxine No. 7+1/540 c. c. antitoxic horse serum (Ldrl. 30).	40	.....do.........................	Very severe symptoms.
			Same serum 68 days old.	
9370	0.23 c. c. toxine No. 7+1/1120 c. c. antitoxic horse serum (NY 306).	61	6 c. c. normal horse (roan) serum subcutaneously.	Dead in 45 minutes.
9447	0.23 c. c. toxine No. 7+1/290 c. c. antitoxic horse serum (PD 08033).	61	.....do.........................	Dead in 85 minutes.
9375	0.23 c. c. toxine No. 7+1/1060 c. c. antitoxic horse serum (NY 306).	61	.....do.........................	Severe symptoms.
			Same serum 91 days old.	
9460	0.23 c. c. toxine No. 7+1/500 c. c. antitoxic horse serum (Ldrl. 11C).	72	6 c. c. normal horse (roan) serum subcutaneously.	Very severe symptoms.
9462	0.23 c. c. toxine No. 7+1/500 c. c. antitoxic horse serum (Ldrl. 15B).	72	.....do.........................	Dead in 40 minutes.
9465	0.23 c. c. toxine No. 7+1/700 c. c. antitoxic horse serum (Ldrl. 13).	72	.....do.........................	Dead in 90 minutes.
			Same serum 118 days old.	
9158	0.23 c. c. toxine No. 7+1/400 c. c. antitoxic horse serum (Cutter 19734).	141	6 c. c. normal horse (roan) serum subcutaneously.	Dead in 50 minutes.
9633	0.23 c. c. toxine No. 7+1/620 c. c. antitoxic horse serum (Alex. A249).	111	.....do.........................	Dead in 40 minutes.
9636	0.23 c. c. toxine No. 7+1/600 c. c. antitoxic horse serum (Alex. A249).	111	.....do.........................	Dead in 45 minutes.

THE EFFECT OF VARIOUS CHEMICAL SUBSTANCES UPON THE TOXICITY OF HORSE SERUM.

Further attempts were made to influence the toxic action of horse serum by treating the guinea pig with various chemicals.

In the following series the sensitized animals were given a subcutaneous injection of various substances the day before the second injection of serum. No favorable influence upon the anaphylactic state was obtained by the substances used, namely, pancreatin, potassium oxalate, pepsin, sodium sulphate, magnesium sulphate, peptone, calcium chlorate, and calcium acetate.

Table No. 12.—*Effect of various chemicals.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
8545	0.24 c. c. toxine 42+1/640 c. c. antitoxic horse serum (Alex. A249).	32	1 gm. pancreatin; next day 0.5 c. c. normal horse (roan) serum into brain.	Dead in 4 minutes.
8553	0.24 c. c. toxine 42+1/680 c. c. antitoxic horse serum (Alex. A249).	32	1 gm. potassium oxalate; next day serum as above.	Dead in 5 minutes.
8552	0.24 c. c. toxine 42+1/680 c. c. antitoxic horse serum (Alex. A249).	32	0.5 gm. pepsin; next day serum as above.	Dead in 2 minutes.
8532	0.24 c. c. toxine 42+1/620 c. c. antitoxic horse serum (Alex. A249).	32	0.1 gm. sodium sulphate; next day serum as above.	Dead in 3 minutes.
8534	0.24 c. c. toxine 42+1/600 c. c. antitoxic horse serum (Alex. A249).	32	0.1 gm. magnesium sulphate; next day serum as above.	Dead in 2 minutes.
8380	0.24 c. c. toxine 42+1/370 c. c. antitoxic horse serum (PD 08033).	32	0.2 gm. peptone; next day serum as above.	Dead in 2 minutes.
8498	0.24 c. c. toxine 42+1/260 c. c. antitoxic horse serum (Cutter 1838).	32	0.1 gm. calcium chlorate; next day serum as above.	Dead in 2 minutes.
8535	0.24 c. c. toxine 42+1/600 c. c. antitoxic horse serum (Alex. A249).	32	0.1 gm. calcium acetate; next day serum as above.	Dead in 3 minutes.

IODINE.

Obermayer and Pick[a] found that when the aromatic radicals of a protein are combined with various substances the protein loses the power to produce precipitins of closely limited specificity for the original species. Their results suggest that the aromatic groups of the molecule are most closely related to the species specificity.[b] This indicates that the striking specificity of proteins of different species depends upon the aromatic groups of the protein molecule, and Vaughan has found evidence that the toxicity of the proteins depends upon these same groups.

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[a] Obermayer, Fr., and Pick, E. P.: Ueber die chemischen Grundlagen der Arteigenschaften der Eiweisskörper. Wiener klin. Woch., vol. 19, 1906, p. 327.

[b] Wells, H. Gideon: The present status of our knowledge of the chemistry of the processes of immunity. Arch. Internal Med., vol. 1, No. 2, February 15, 1908, p. 262.

Fleischmann[a] also found that tryptic digestion destroys this characteristic species specificity.

We made several tests to determine the effect of iodine upon the toxic action of horse serum, and it so turned out in the preliminary experiments that the symptoms appeared to be profoundly modified, in the sense that they were either delayed or inhibited. We therefore tested a large number of guinea pigs to determine this point, but found that so far as the toxicity of horse serum was concerned at the second injection it is not appreciably modified by the iodine.

In the following series the iodine was added to horse serum in the proportion of 1.5 gm. per 25 c. c. of serum and 3 gm. potassium iodide to aid solution.

TABLE No. 13.—*Iodine added to serum.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9826	0.23 c. c. toxine No. 7+1/250 c. c. antitoxic horse serum (P. I. Bur.).	42	6 c. c. normal horse (Frank) serum and iodine subcutaneously.	Dead in 50 minutes.
9837	0.23 c. c. toxine No. 7+1/210 c. c. antitoxic horse serum (Swiss USA).	42	.....do...........................	Dead in 80 minutes.
9830	0.23 c. c. toxine No. 7+1/390 c. c. antitoxic horse serum (Mul. 2539).	42	.....do...........................	Severe symptoms.
9832A	0.23 c. c. toxine No. 7+1/480 c. c. antitoxic horse serum (Mul. 2539).	42	.....do...........................	Slight symptoms.
725	0.0004 c. c. tetanus toxine A+0.0075 c. c. antitetanic serum (Standard).	42	.....do...........................	Mild symptoms.
9778	0.23 c. c. toxine No. 7+1/320 c. c. antitoxic horse serum (Strn. 1351).	53	6 c. c. normal horse serum (Teddy) and iodine subcutaneously.	Dead in 65 minutes.
9713	0.23 c. c. toxine No. 7+1/400 c. c. antitoxic horse serum (Scher. II).	66	.....do...........................	Slight symptoms.
9743	0.23 c. c. toxine No. 7+1/170 c. c. antitoxic horse serum (Strn. 1110).	53	.....do...........................	Slight symptoms.
9182	0.23 c. c. toxine No. 7+1/140 c. c. antitoxic horse serum (Mul. 2352).	103	6 c. c. normal horse (roan) serum and iodine intraperitoneally.	Dead in 30 minutes.
9187	0.23 c. c. toxine No. 7+1/320 c. c. antitoxic horse serum (Mul. 2362).	103	.....do...........................	Dead in 45 minutes.
9189	0.23 c. c. toxine No. 7+1/300 c. c. antitoxic horse serum (Mul. 2433).	103	.....do...........................	Dead in 15 minutes.
9783	0.23 c. c. toxine No. 7+1/300 c. c. antitoxic horse serum (Strn. 1110).	53	6 c. c. normal horse (roan) serum and iodine subcutaneously.	Mild symptoms.

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[a] Zeit. f. klin. Med., vol. 59, 1906, p. 515.

TABLE NO. 13.—*Iodine added to serum*—Continued.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9730	0.23 c. c. toxine No. 7+1/170 c. c. antitoxic horse serum (Strn. 1110).	53	6 c. c. normal horse (roan) serum and iodine subcutaneously.	Mild symptoms.
8551	0.23 c. c. toxine No. 7+1/700 c. c. antitoxic horse serum (Alex. A249).	237	.....do...........................	Mild symptoms.
9554	0.23 c. c. toxine No. 7+1/320 c. c. antitoxic horse serum (Well. 7L841).	73	.....do...........................	Dead in 80 minutes.

TABLE NO. 14.—*Iodine injected separately.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9782	0.23 c. c. toxine No. 7+1/300 c. c. antitoxic horse serum (Strn. 1351).	53	Iodine (5 c. c. Gram's solution) subcutaneously; next day 0.2 c. c. normal horse (roan) serum into brain.	Dead in 5 minutes.
9752	0.23 c. c. toxine No. 7+1/180 c. c. antitoxic horse serum (Strn. 1110).	53	Iodine (3 c. c. Gram's solution) subcutaneously; 1 hour later 6 c. c. normal horse (Teddy) serum intraperitoneally.	Marked symptoms.
9772	0.23 c. c. toxine No. 7+1/280 c. c. antitoxic horse serum (Strn. 1351).	53	Iodine (6 c. c. Gram's solution) subcutaneously; 1 hour later normal horse serum (Teddy) intraperitoneally.	Marked symptoms.
9657	0.23 c. c. toxine No. 7+1/340 c. c. antitoxic horse serum (PD 08022).	64	Iodine (5 c. c. Gram's solution) subcutaneously; 1 hour later 0.2 c. c. normal horse (roan) serum into brain.	Dead in 3 minutes.
9781	0.23 c. c. toxine No. 7+1/310 c. c. antitoxic horse serum (Strn. 1351).	53	Iodine (5 c. c. Gram's solution); 1 hour later 0.1 c. c. same horse serum into brain.	Dead in 3 minutes.

TABLE NO. 15.—*Controls for tables Nos. 13 and 14.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9836	0.23 c. c. toxine No. 7+1/170 c. c. antitoxic horse serum (Swiss).	42	6 c. c. normal horse serum (Frank) subcutaneously.	Very severe symptoms.
9832	0.23 c. c. toxine No. 7+1/570 c. c. antitoxic horse serum (Mul. 2539).	42	.....do...........................	Very severe symptoms.
9773	0.23 c. c. toxine No. 7+1/280 c. c. antitoxic horse serum (Strn. 1351).	52	6 c. c. normal horse serum (Teddy) subcutaneously.	Severe symptoms.
9770	0.23 c. c. toxine No. 7+1/290 c. c. antitoxic horse serum (Strn. 1351).	52	.....do...........................	Dead in 40 minutes.
9780	0.23 c. c. toxine No. 7+1/310 c. c. antitoxic horse serum (Strn. 1351).	52	.....do...........................	Dead in 60 minutes.

NITRITES.

A few further tests were made to determine the relation of methemaglobin producing substances, such as the nitrites, upon the symptoms. The following two guinea pigs were given a subcutaneous injection of sodium nitrite. In thirty minutes the exposed mucous membranes appeared distinctly blue, and the guinea pigs were then tested for susceptibility.

Controls showed that the quantity of nitrite used was not sufficient in itself to kill the guinea pigs.

TABLE No. 16.—*Sodium nitrite.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9173	0.23 c.c. toxine No. 7+1/360 c.c. antitoxic horse serum (Mul. 2439).	106	0.03 gm. sodium nitrite; 30 minutes later 1 c. c. normal horse (roan) serum intraperitoneally.	Dead in 15 minutes.
9168	0.23 c.c. toxine No. 7+1/520 c.c. antitoxic horse serum (Mem. Inst.).	106	0.025 gm. sodium nitrite; 30 minutes later 1 c. c. normal horse (roan) serum subcutaneously.	Dead in 60 minutes

ETHER.

Besredka[a] reported some interesting observations concerning the prevention of anaphylaxis by ether narcosis. He stated that if sensitive guinea pigs are etherized to the stage of complete relaxation, and while in this state injected intracerebrally with normal horse serum and the administration of ether continued a short while, (if the narcosis is well conducted) the animals continue to sleep after the injection, and at the end of about half an hour awake without presenting the least symptoms of anaphylaxis. If the guinea pig is tested on the following day it will be found to be immune.

Of eight guinea pigs upon which we tried this experiment with ether seven died, we believe, from the effects of the second injection of horse serum. It is our belief that pig No. 9581, which recovered, had masked symptoms while under the influence of the ether and probably would not have died anyhow, for we have a certain number of recoveries from the intracerebral injections of 0.2 c. c. of horse serum. It is true, however, that the narcosis masks the symptoms.

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[a] Annales de l'Institut Pasteur, Dec. 25, 1907, vol. 21, no. 12, p. 957.

The difference in our results may be accounted for by the difference in toxicity of the French and the American serums, or by differences in susceptibility of the animals used.

TABLE No. 17.—*Ether.*

G. P. No.	Treatment.	Result.
9419	0.23 c. c. toxine No. 7+1/440 c. c. antitoxic horse serum (Alex. A276). 55 days later tested as follows: The animal was first trephined, then deeply narcotized with ether, and in the stage of complete relaxation injected with 0.25 c. c. antitoxic horse serum (Mul. 2295) into the brain. The etherization was continued several minutes longer.	Died.
9376	0.23 c. c. toxine No. 7+1/1060 c. c. antitoxic horse serum (NY 306). 62 days later etherized and tested as above with 0.1 c. c. same serum.	Died.
9374	0.23 c. c. toxine No. 7+1/1080 c. c. antitoxic horse serum (NY 306). 62 days later etherized and tested as above with 0.25 c. c. same serum.	Died.
9175	0.23 c. c. toxine No. 7+1/540 c. c. antitoxic horse serum (Mul. 2439). 106 days later etherized and tested with 0.2 c. c. normal horse (roan) serum into brain.	Died in 5 minutes.
9174	0.23 c. c. toxine No. 7+1/450 c. c. antitoxic horse serum (Mul. 2429). 106 days later etherized and tested with 0.2 c. c. normal horse (roan) serum into brain.	Died in 5 minutes.
9678	0.23 c. c. toxine No. 7+1/320 c. c. antitoxic horse serum (Alex. A245.) 73 days later etherized and tested with 0.2 c. c. normal horse (Frank) serum into brain.	Died in 10 minutes.
9581	0.23 c. c. toxine No. 7+1/280 c. c. antitoxic horse serum (Alex. A245). 73 days later etherized and tested with 0.2 c. c. normal horse (Frank) serum into brain.	Recovered.
	Next day 6 c. c. normal horse (Frank) serum intraperitoneally..............	No symptoms.
9582	0.23 c. c. toxine No. 7+1/280 c. c. antitoxic horse serum (Alex. A245). 65 days later etherized and tested with 0.2 c. c. antitoxic horse serum (Lyons) into brain.	Died in few minutes.

ATTEMPTS TO NEUTRALIZE HORSE SERUM WITH THE BRAIN AND BLOOD OF SENSITIZED GUINEA PIGS.

We made further attempts to neutralize the toxic action of horse serum by first treating it with sensitized guinea pig serum and also with the brain substance of sensitized guinea pigs. These attempts all proved futile.

In the following experiments normal horse serum was mixed with equal parts of sensitized guinea pig serum, and kept at room temperature twenty-four hours.

The brain of a sensitive guinea pig was removed under aseptic precautions, mixed with about 20 c. c. of normal horse serum, ground up in a mortar, kept at room temperature for twenty-four hours, and then strained.

TABLE NO. 18.—*Action of guinea pig serum and brain.*

G. P. No.	First injection.	Interval in days.	Second injection	Result.
9337	0.23 c. c. toxine No. 7+1/1020 c. c. antitoxic horse serum (NY 305).	96	0.2 c. c. mixture of normal horse (roan) and sensitized guinea pig (9558) serum into brain.	Dead in 4 minutes.
9340	0.23 c. c. toxine No. 7+1/1000 c. c. antitoxic horse serum (NY 305).	96	0.1 c. c. mixture of normal horse (roan) and sensitized guinea pig (9558) serum into brain.	Very severe symptoms.
9336	0.23 c. c. toxine No. 7+1/1040 c. c. antitoxic horse serum (NY 305).	96	0.2 c. c. mixture of normal horse (roan) serum and brain of sensitized guinea pig (9558) into brain.	Dead in 5 minutes.
9338	0.23 c. c. toxine No. 7+1/1020 c. c. antitoxic horse serum (NY 305).	96	0.1 c. c. mixture of normal horse (roan) serum and brain of sensitized guinea pig (9558) into brain.	Marked symptoms.

PART 4.—SPECIFICITY.

SPECIFIC NATURE OF "ANAPHYLACTIN."

In a previous publication[a] on the specific nature of anaphylaxis we demonstrated that guinea pigs may be in a condition of anaphylaxis to three protein substances at the same time. Hypersusceptibility to each protein is manifested by a second injection of the corresponding protein. The three reactions may be obtained in the same guinea pig within a short space of time and are as distinct and specific as three separate infectious diseases.

We now bring forward experimental data proving that the substance in the blood serum of sensitized guinea pigs, known as anaphylactin, is also specific in the same sense. In the following table it will be seen that by transferring the blood serum of guinea pigs sensitized to horse serum, egg white, and milk three separate and distinct reactions were obtained in the guinea pig into which this serum was transferred:

TABLE No. 19.—*The specific nature of anaphylactin.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9619	0.23 c. c. toxine No 7+1/600 c. c. antitoxic horse serum (Alex. A249) subcutaneously. 56 days later 1 c. c. cow's milk subcutaneously. 1 day later 1 c. c. egg white subcutaneously. 22 days later bled; blood defibrinated centrifuged, and serum used to inject G. P. No. 9619A.			
9619A	7 c. c. serum of G. P. 9619 subcutaneously.	2	6 c. c. milk intraperitoneally....	Mild symptoms.
			2 days later 0.2 c. c. normal horse (Teddy) serum into brain.	Dead in 5 minutes.
9620	Same treatment as G. P. No. 9619.			
9620A	8 c. c. serum of G. P. 9620 subcutaneously.	2	6 c. c. milk intraperitoneally....	Slight symptoms.
			2 days later 3 c. c. egg white intraperitoneally.	Mild symptoms.
			2 hours later 0.2 c. c. normal horse (Teddy) serum into brain.	Dead in 4 minutes.
9621	0.23 c. c. toxine No. 7+1/580 c. c. antitoxic horse serum (Alex. A249). Subsequent treatment same as G. P. 9619.			
9621A	6.5 c. c. serum of G. P. 9621 subcutaneously.	2	6 c. c. milk intraperitoneally....	Slight symptoms.
			2 days later 6 c. c. saturated solution egg white intraperitoneally.	Marked symptoms.
			2 hours later 0.2 c. c. normal horse (Teddy) serum into brain.	Very severe symptoms.
9624	0.23 c. c. toxine No. 7+1/560 c. c. antitoxic horse serum (Alex. A249). Subsequent treatment same as G. P. 9619.			
9624A	7 c. c. serum of G. P. 9624 subcutaneously.	2	6 c. c. milk intraperitoneally....	Slight symptoms.
			2 days later 0.2 c. c. normal horse (Teddy) serum into brain.	Dead in 5 minutes.

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[a] Rosenau, M. J., and Anderson, John F.: The specific nature of anaphylaxis. Jour. Infec. Dis., vol. 4, No. 4, Nov., 1907, pp. 552–557.

EFFECT OF IODINE UPON SPECIFICITY.

In the following series we not only tested guinea pigs treated with horse serum to find out whether they react after an interval to chicken serum, and vice versa, but also to ascertain whether the presence of iodine in the sensitizing dose modifies the specific nature of the reaction. The results plainly show that under the conditions of the experiment the iodine had no modifying action upon the power of these serums to sensitize against a second injection of homologous serum and also had no power to impair the specific nature of the phenomenon.

Table No. 20.—*Effect of iodine on specificity.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1613	1 c. c. 0.01 per cent chicken serum +iodine subcutaneously.	19	0.2 c. c. chicken serum into brain.	Severe symptoms.
1604	.....do...........................	19	.....do.......................	Severe symptoms.
1615	2 c. c. 0.01 per cent chicken serum +iodine subcutaneously.	19	0.2 c. c. normal horse (Teddy) serum into brain.	No symptoms.
1616	.....do...........................	19	.....do.......................	No symptoms.
1617	1 c. c. 0.01 per cent chicken serum +iodine subcutaneously.	19	.....do.......................	No symptoms.
1618	2 c. c. 0.01 per cent normal horse (Frank) serum +iodine subcutaneously.	19	.....do.......................	Severe symptoms.
1619	1 c. c. 0.01 per cent normal horse (Frank) serum +iodine subcutaneously.	19	.....do.......................	Dead in 4 minutes
1620	.....do...........................	19	0.2 c. c. chicken serum into brain.	No symptoms.
1621	2 c. c. 0.01 per cent normal horse (Frank) serum +iodine subcutaneously.	19	.....do.......................	No symptoms.
1622	1 c. c. 0.01 per cent normal horse (Frank) serum +iodine subcutaneously.	19	.....do.......................	No symptoms.

RELATION BETWEEN MILKS OF VARIOUS SPECIES.

HUMAN VERSUS COW MILK.

Eight guinea pigs were sensitized by the subcutaneous injection of 1 c. c. of human milk. After an appropriate interval they were tested with cow's milk without response.

A short time later they were again tested with human milk. This time most of them showed severe symptoms.

This series again indicates not only the specific nature of the anaphylactic reaction, but suggests differences between the protein matter in human and in cow's milk.

Table No. 21.—*Human versus cow milk.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1375	1 c. c. human milk subcutaneously.	37	6 c. c. cow's milk intraperitoneally. 2 days later 10 c. c. human milk subcutaneously.	No symptoms. No symptoms(?).
1376	.....do	37	6 c. c. cow's milk subcutaneously... 4 hours later 10 c. c. human milk intraperitoneally.	No symptoms. Very severe symptoms.
1377	.....do	37	6 c. c. cow's milk subcutaneously... 4 hours later 10 c. c. human milk intraperitoneally.	No symptoms. Very severe symptoms.
1378	.....do	37	6 c. c. cow's milk intraperitoneally.. 2 days later 10 c. c. human milk intraperitoneally.	No symptoms. No symptoms(?).
1401	.....do	24	6 c. c. cow's milk intraperitoneally.. 1 day later 5 c. c. human milk intraperitoneally.	No symptoms. Very severe symptoms.
1402	.....do	24	6 c. c. cow's milk intraperitoneally.. 1 day later 5 c. c. human milk intraperitoneally.	No symptoms. Very severe symptoms.
1403	.....do	24	6 c. c. cow's milk intraperitoneally.. 1 day later 5 c. c. human milk intraperitoneally.	No symptoms. Very severe symptoms.
1404	.....do	24	6 c. c. cow's milk intrapertioneally.. 1 day later 5 c. c. human milk intraperitoneally.	No symptoms. Very severe symptoms.

Table No. 22.—*Sheep milk versus cow's milk.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1450	1 c. c. sheep's milk subcutaneously.	26	6 c. c. cow's milk subcutaneously.	No symptoms.
1451	.....do	26	.....do	No symptoms.
1452	.....do	26	6 c. c. cow's milk intraperitoneally.	Marked symptoms.
1453	.....do	26	.....do	Severe symptoms.
1454	.....do	26	.....do	Slight symptoms.
1455	.....do	26	.....do	Dead in 25 minutes.

Table No. 23.—*Dog milk versus cow's milk.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1600	0.5 c. c. dog milk subcutaneously...	17	6 c. c. cow's milk intraperitoneally.	No symptoms.
1601	.....do	17	.....do	No symptoms.
1602	.....do	17	.....do	No symptoms.
1603	1 c. c. dog milk subcutaneously.....	17	.....do	No symptoms.
1604	.....do	17	.....do	No symptoms.
1605	.....do	19	.....do	No symptoms.
1606	.....do	19	.....do	No symptoms.
1607	.....do	19	.....do	No symptoms.

In the following series the dog milk was first treated with iodine and potassium iodide before it was injected subcutaneously.

TABLE No. 24.—*Dog milk+iodine versus cow's milk.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1608	0.5 c. c. dog milk+iodine subcutaneously.	17	6 c. c. cow's milk intraperitoneally.	No symptoms.
1609	.....do	17	.....do	No symptoms.
1610	.....do	17	.....do	No symptoms.
1611	1 c. c. dog milk+iodine subcutaneously.	17	.....do	No symptoms.
1612	.....do	17	.....do	No symptoms.

RELATION BETWEEN EGG ALBUMENS OF VARIOUS SPECIES.

TABLE No. 25.—*Hen egg white versus duck egg white.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1408	1 c. c. saturated solution hen egg white in salt solution (0.85) subcutaneously.	21	10 c. c. saturated solution duck egg white in salt solution (0.85) intraperitoneally.	Mild symptoms.
1409	.....do	21	.....do	Mild symptoms.
1410	.....do	21	.....do	Mild symptoms.
1411	.....do	21	.....do	No symptoms.

TABLE No. 26.—*Duck egg white versus hen egg white.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1413	1 c. c. saturated solution duck egg white in salt solution (0.85) subcutaneously.	21	10 c. c. saturated solution hen egg white in salt solution (0.85) intraperitoneally.	Dead in 30 minutes.
1414	.....do	21	.....do	Dead in 35 minutes.
1415	.....do	21	.....do	Very severe symptoms.
1416	.....do	21	.....do	Very severe symptoms.
1417	.....do	21	.....do	Very severe symptoms.

Table No. 27.—*Guinea hen egg white versus hen and crane egg white.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
2040	1 c. c. saturated solution guinea hen egg white in salt solution (0.85) subcutaneously.	20	6 c. c. saturated solution hen egg white in salt solution (0.85) intraperitoneally.	Dead in 13 minutes.
2041	.....do	20	.....do	Very severe symptoms.
2042	.....do	20	.....do	Very severe symptoms.
2043	.....do	20	6 c. c. saturated solution of crane egg white in salt solution (0.85) intraperitoneally.	Marked symptoms.
2044	.....do	20	.....do	Marked symptoms.

Note.—The crane egg used in these experiments was from a Demoiselle crane and kindly furnished us by Dr. Frank Baker.

Table No. 28.—*Pigeon egg white versus hen and crane egg white.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
2035	1 c. c. saturated solution pigeon egg white in salt solution (0.85) subcutaneously.	21	6 c. c. saturated solution hen egg white in salt solution (0.85) intraperitoneally.	No symptoms.
2036	.....do	21	.....do	No symptoms.
2037	.....do	21	.....do	No symptoms.
2038	.....do	21	6 c. c. saturated solution of crane egg white in solt solution (0.85) intraperitoneally.	Very severe symptoms.
2039	.....do	21	.....do	Marked symptoms.

Table No. 29.—*Goose egg white versus hen and turkey egg white.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
2030	1 c.c. saturated solution goose egg white in salt solution (0.85) subcutaneously.	38	6 c. c. saturated solution hen egg white in salt solution (0.85) intraperitoneally.	Marked symptoms.
2031	.....do	38	.....do	Very severe symptoms.
2032	.....do	38	.....do	Marked symptoms.
2033	.....do	38	6 c. c. saturated salt solution turkey egg white in salt solution (0.85) intraperitoneally.	Very severe symptoms.
2034	.....do	38	.....do	Severe symptoms.

TABLE NO. 30.—*Turkey egg white versus hen and crane egg white.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
2025	1 c. c. saturated solution turkey egg white in salt solution (0.85) subcutaneously.	35	6 c. c. saturated solution of hen egg white in salt solution (0.85) intraperitoneally.	Very severe symptoms.
2026	.....do	35	.....do	Dead in 70 minutes.
2027	.....do	35	.....do	Dead in 70 minutes
2028	.....do	35	6 c. c. saturated solution of crane egg white in salt solution (0.85) intraperitoneally.	Severe symptoms.
2029	.....do	35	.....do	Severe symptoms.
2030	.....do	35	6 c. c. saturated solution of turkey egg white in salt solution (0.85) intraperitoneally.	Dead in 20 minutes.

PART 5.—ANAPHYLACTIN.

THE PRESENCE OF ANAPHYLACTIN DURING THE PERIOD OF INCUBATION.

It is of some interest to determine just when the substance called *anaphylactin* by Gay and Southard appears in the blood of a sensitized guinea pig, particularly whether its presence may be demonstrated during the period of incubation.

A series of guinea pigs was therefore sensitized by the subcutaneous injection of 1/100 c. c. of normal horse serum. On each succeeding day two guinea pigs of this series were bled and the serum obtained by whipping and centrifugation. This serum was then injected into normal pigs. In twenty-four hours these pigs were tested by the injection of 6 c. c. of horse serum intraperitoneally. In the latter part of the series forty-eight hours were allowed to elapse before the pigs were tested.

It will be seen from Tables 31 and 32, which give our work on this factor in detail, that no indication of anaphylactin appeared in the blood of sensitized guinea pigs until the tenth day.

TABLE No. 31.—*Anaphylactin during the period of incubation.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1343A	2 c. c. subcutaneously serum G. P. 1343, which was bled *1 day* after subcutaneous injection of 0.01 c. c. normal horse serum.	1	0.25 c. c. normal horse serum into brain.	No symptoms.
1343B	.....do	1	5 c. c. normal horse serum intraperitoneally.	No symptoms.
1344A	4 c. c. serum G. P. 1344, which was bled *2 days* after injection of 0.01 c. c. normal horse serum.	1	6 c. c. normal horse serum intraperitoneally.	No symptoms.
1345A	3 c. c. serum G. P. 1345, which was bled *3 days* after injection of 0.01 c. c. normal horse serum.	1	.....do	No symptoms.
1345B	.....do	1	.....do	No symptoms.
1346A	2.5 c. c. serum G. P. 1346, which was bled *4 days* after injection of 0.01 c. c. normal horse serum.	1	.....do	No symptoms.
1346B	.....do	1	.....do	No symptoms.

TABLE No. 31.—*Anaphylactin during the period of incubation*—Continued.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1347A	3 c. c. serum G. P. 1347, which was bled *5 days* after injection of 0.01 c. c. normal horse serum.	1	6 c. c. normal horse serum intraperitoneally.	No symptoms.
1347B	.....do	1	.....do	No symptoms.
1348A	5 c. c. serum G. P. 1348, which was bled *6 days* after injection of 0.01 c. c. normal horse serum.	1	.....do	No symptoms.
1348B	.....do	1	.....do	No symptoms.
1349A	4 c. c. serum G. P. 1349, which was bled *7 days* after injection of 0.01 c. c. normal horse serum.	1	.....do	No symptoms.
1350A	5 c. c. serum G. P. 1350, which was bled *8 days* after injection of 0.01 c. c. normal horse serum.	1	.....do	No symptoms.
1351A	6 c. c. serum G. P. 1351, which was bled *9 days* after injection of 0.01 c. c. normal horse serum.	1	.....do	No symptoms.
1353A	8 c. c. serum G. P. 1353, which was bled *10 days* after injection of 0.01 c. c. normal horse serum.	2	.....do	Marked symptoms.
1354A	6.5 c. c. serum G. P. 1354, which was bled *11 days* after injection of 0.01 c. c. normal horse serum.	1	.....do	Slight symptoms.
1355A	6.5 c. c. serum G. P. 1355, which was bled *12 days* after injection of 0.01 c. c. normal horse serum.	1	.....do	Slight symptoms.
1355B	1 c. c. same serum	2	.....do	Marked symptoms.
1356A	3 c. c. serum G. P. 1356, which was bled *13 days* after injection of 0.01 c. c. normal horse serum.	1	.....do	No symptoms.
1356B	2.5 same serum	2	.....do	Mild symptoms.
1357A	3 c. c. serum G. P. 1357, which was bled *14 days* after injection of 0.01 c. c. normal horse serum.	1	.....do	Mild symptoms.
1357B	2.5 same serum	3	.....do	Severe symptoms.

The first indication of anaphylactin appeared in the above series on the tenth day, that is, just about the time necessary to render guinea pigs sensitive. It is also evident that forty-eight hours is a better interval than twenty-four for the purpose of demonstrating the presence of anaphylactin in guinea-pig serum.

Our interest in this subject led us to sensitize another series of guinea pigs with 1/100 c. c. normal horse serum subcutaneously, but to test for the presence of anaphylactin by injections into the brain.

TABLE No. 32.—*Anaphylactin during the period of incubation.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1380A	6 c. c. subcutaneously serum G. P. 1380, which was bled *5 days* after injection of 0.01 c. c. normal horse serum.	2	0.2 c. c. normal horse serum into brain.	No symptoms.
1381A	4 c. c. intraperitoneally serum G. P. 1381, which was bled *6 days* after injection of 0.01 c. c. normal horse serum.	2	....do....	No symptoms.
1382A	4 c. c. intraperitoneally serum G. P. 1382, which was bled *6 days* after injection of 0.01 c. c. normal horse serum.	2	....do....	No symptoms.
1383A	5 c. c. intraperitoneally serum G. P. 1383, which was bled *7 days* after injection of 0.01 c. c. normal horse serum.	2	....do....	No symptoms.
1384A	5 c. c. intraperitoneally serum G. P. 1384, which was bled *7 days* after injection of 0.01 c. c. normal horse serum.	2	....do....	No symptoms.
1385A	5 c. c. intraperitoneally serum G. P. 1385, which was bled *8 days* after injection of 0.01 c. c. normal horse serum.	2	....do....	No symptoms.
1386A	5 c. c. intraperitoneally serum G. P. 1386, which was bled *8 days* after injection of 0.01 c. c. normal horse serum.	2	....do....	No symptoms.
1387A	5 c. c. intraperitoneally serum G. P. 1387, which was bled *9 days* after injection of 0.01 c. c. normal horse serum.	2	....do....	Slight symptoms.
1388A	5 c. c. intraperitoneally serum G. P. 1388, which was bled *9 days* after injection of 0.01 c. c. normal horse serum.	2	....do....	Slight symptoms.
1389A	6 c. c. intraperitoneally serum G. P. 1389, which was bled *10 days* after injection of 0.01 c. c. normal horse serum.	2	....do....	Marked symptoms.
1390A	3.5 c. c. intraperitoneally serum G. P. 1390, which was bled *10 days* after injection of 0.01 c. c. normal horse serum.	2	....do....	Mild symptoms.
1391A	5 c. c. intraperitoneally serum G. P. 1391, which was bled *11 days* after injection of 0.01 c. c. normal horse serum.	2	....do....	Mild symptoms.
1391B	Hemaglobin intraperitoneally of G. P. 1391.	2	....do....	No symptoms.
1391C	Red corpuscles of G. P. 1391 intraperitoneally.	2	....do....	No symptoms.

In the above series the anaphylactin appeared in the blood of sensitized guinea pigs on the ninth day.

ANAPHYLACTIN IN MAN AND OTHER ANIMALS.

The presence of anaphylactin having been demonstrated in the blood serum of sensitized guinea pigs, it is interesting to know whether man and other animals that have received a previous injection of horse serum also contain a similar substance.

So far as may be judged from the following limited experiments upon the subject, we have been unable to demonstrate a similar property in the blood serum of man, the monkey, rabbit, and the cat when tested upon guinea pigs.

TABLE NO. 33.—*Anaphylactin in man, monkey, rabbit, and cat.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
	Man.			
1310	1 c. c. normal human serum (CWC) subcutaneously.	44	6 c. c. normal horse (roan) serum intraperitoneally.	Slight symptoms.
280	5 c. c. human serum (LLL). 7 years prior this man had been injected with 12 c. c. antitoxic horse serum.	1¾	.....do........................	No symptoms.
1311	0.4 c. c. human serum (JWT). 1 year prior this man had been injected with 5,000 units antitoxic horse serum.	34	.....do........................	Slight symptoms.
1312	1.5 human serum (WWM). 2½ years prior this man had been injected with 1,500 units antitoxic horse serum.	36	.....do........................	Marked symptoms.
1313	0.5 c. c. human serum (MJR). 3 months prior this man had been injected with 10 c. c. antitetanic horse serum.	36	.....do........................	Mild symptoms.
1314	1.5 c. c. human serum (MJR). 3 months prior this man had been injected with 10 c. c. antitetanic horse serum.	36	.....do........................	Slight symptoms.
1379	10 c. c. human serum (WWM). 2½ years prior this man had been injected with 1,500 units antitoxic horse serum.	2½	0.2 c. c. normal horse (roan) serum into brain.	No symptoms.
	Monkey.			
290	3.5 c. c. monkey (Billy) serum. 2 years prior this monkey had been injected with 2 (?) c. c. normal horse serum.	1⅚	6 c. c. normal horse (roan) serum intraperitoneally.	No symptoms.
290A	3 c. c. monkey (Billy) serum. 2 years prior this monkey had been injected with 2 (?) c. c. normal horse serum.	1⅚	.....do........................	No symptoms.

TABLE No. 33.—*Anaphylactin in man, monkey, rabbit, and cat*—Continued.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
	Cat.			
285	3 c. c. cat serum. 41 days prior this cat had been injected with 2 c. c. normal horse serum.	1⅚	6 c. c. normal horse (roan) serum intraperitoneally.	No symptoms.
	Rabbit.			
1377	8 c. c. rabbit serum. This rabbit had been frequently injected with normal horse serum.	2½	....do....................	No symptoms.
1378	.....do..............................	2½	0.2 c. c. normal horse (roan) serum into brain.	No symptoms

It will be noticed that a few of these guinea pigs showed symptoms, but in every case an interval of at least two weeks elapsed between the first and the second injections. The slight reactions obtained were probably those which occur when serums of different species are used at the first and the second injections (heterologous serums, see Hyg. Lab. Bull. 29, p. 55, and Bull. 36, p. 25).

ANAPHYLACTIN IN IMMUNE GUINEA PIGS.

We submit some further work bearing upon the question whether guinea pigs "immunized" against the phenomenon of anaphylaxis are in a refractory state or have returned to the normal or are really immune.

We first made a few experiments to determine whether guinea pigs immunized by repeated injections of small quantities of horse serum contain anaphylactin in their blood. In this series the guinea pigs received ten injections of 2 c. c. normal horse serum subcutaneously covering a period of seventeen days. Eighteen days following the last injection the pigs were first tested for immunity and then bled.

It developed from the following six guinea pigs that ten repeated subcutaneous injections (of 2 c. c. each) during the course of seventeen days were not sufficient to completely immunize the guinea pigs, for those tested developed slight symptoms. Indications of anaphylactin were demonstrated in the blood serum of five of the six pigs of this series. However, it must be noted that when bled these animals were certainly immune from the last treatment, as we and others have shown that when a sensitive guinea pig responds to a second injection immunity is quickly established. (See G. P. No. 671, Table 36.)

TABLE NO. 34.—*Anaphylactin in immune guinea pigs.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1370A	6 c. c. serum G. P. 1370, which had received 10 subcutaneous injections 2 c. c. normal horse serum in a period of 17 days.	2	6 c. c. normal horse serum intraperitoneally.	Mild symptoms.
1373A	6 c. c. serum G. P. 1373, which had same treatment as 1370.	2	0.2 c. c. normal horse serum into brain.	Mild symptoms.
1369A	6 c. c. serum G. P. 1369, which had same treatment as 1370.	2	6 c. c. normal horse serum intraperitoneally.	Marked symptoms.
1371A	6 c. c. serum G. P. 1371, which had same treatment as 1370.	2	0.2 c. c. normal horse serum into brain.	Slight symptoms.
1374A	6 c. c. serum G. P. 1374, which had same treatment as 1370.	2	6 c. c. normal horse serum intraperitoneally.	Marked symptoms.
1372A	6 c. c. serum G. P. 1372, which had same treatment as 1370.	2	0.2 c. c. normal horse serum into brain.	No symptoms.
671	0.0006 c. c. toxin A+1/1538 c. c. antitoxic horse serum (PD 09755).	55	2 c. c. antitoxic horse serum (Natl. IX) intraperitoneally.	Very severe symptoms.
			Next day 0.2 c. c. normal horse (roan) serum into brain. 30 minutes later bled for serum.	No symptoms.
671A	5 c. c. serum of G. P. 671T intraperitoneally.	2	0.2 c. c. normal horse (Frank) serum into brain.	No symptoms.
666	0.0006 c. c. toxin A + .0002 gm. antitetanic serum (Tizzoni).	55	2 c. c. antitoxic horse serum (Natl. IX) intraperitoneally. Next day bled for serum.	Very severe symptoms.
666A	6 c. c. serum of G. P. 666T intraperitoneally.	2	0.2 c. c. normal horse (Frank) serum into brain.	No symptoms

The following series of guinea pigs is a more conclusive test that anaphylactin may be demonstrated in the blood serum of immune guinea pigs.

Guinea pigs were first immunized by repeated subcutaneous injections of 5 c. c. normal horse serum. The pigs received from 6 to 12 such injections, amounting to 30 to 60 c. c. About eight months after this treatment the pigs were tested by intracerebral injections, to which some responded with slight symptoms. Two of them showed no symptoms at all. While all the pigs were not completely immunized by the first treatment of subcutaneous injections, they were certainly rendered immune by the second treatment, given eight months later. Two hours after the second treatment the guinea pigs were bled, the blood centrifugated, the clear serum pipetted off and injected subcutaneously into normal guinea pigs. These normal pigs so treated were tested two days later by intracerebral injections, and all of them showed symptoms.

TABLE No. 35.—*Anaphylactin in immune guinea pigs.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1180	6 injections, each 5 c. c. normal horse (roan) serum subcutaneously, covering period of 18 days.	249 after last injection	0.2 c. c. normal horse (roan) serum into brain. 2 hours later bled for serum.	Slight symptoms
1180A	9 c. c. serum G. P. 1180 subcutaneously.	2	0.15 c. c. normal horse (Teddy) serum into brain.	Slight symptoms.
1181	6 injections, each 5 c. c. normal horse (roan) serum, in 18 days.	249	0.2 c. c. normal horse (roan) serum into brain. 2 hours later bled for serum.	No symptoms.
1181A	8 c. c. serum G. P. 1181 subcutaneously.	2	0.15 c. c. normal horse (Teddy) serum into brain.	Marked symptoms.
1182	6 injections, each 5 c. c. normal horse (roan) serum, in 18 days.	249	0.2 c. c. normal horse (roan) serum into brain. 2 hours later bled for serum.	Slight symptoms.
1182A	8 c. c. serum G. P. 1182 subcutaneously.	2	0.15 c. c. normal horse (Teddy) serum into brain.	Marked symptoms.
1183	6 injections, each 5 c. c. normal horse (roan) serum, in 18 days.	249	0.2 c. c. normal horse (roan) serum into brain. 2 hours later bled for serum.	Slight symptoms.
1183A	12 c. c. serum G. P. 1183 subcutaneously.	2	0.15 c. c. normal horse (Teddy) serum into brain.	Slight symptoms.
1186	7 injections, each 5 c. c. normal horse (roan) serum, in 21 days.	246	0.2 c. c. normal horse (roan) serum into brain. 2 hours later bled for serum.	Slight symptoms.
1186A	8 c. c. serum G. P. 1186 subcutaneously.	2	0.15 c. c. normal horse (Teddy) serum into brain.	Slight symptoms.
1187	8 injections, each 5 c c. normal horse (roan) serum, in 25 days	242	0.2 c. c. normal horse (roan) serum into brain. 2 hours later bled for serum.	No symptoms.
1187A	9 c. c. serum G. P. 1187 subcutaneously.	2	0.15 c. c. normal horse (Teddy) serum into brain.	Slight symptoms.
1188	9 injections, each 5 c. c. normal horse (roan) serum, in 28 days.	239	0.2 c. c. normal horse (roan) serum into brain. 2 hours later bled for serum.	Slight symptoms.
1188A	8 c. c. serum G. P. 1188 subcutaneously.	2	0.15 c. c. normal horse (Teddy) serum into brain.	Slight symptoms.
1189	10 injections, each 5 c. c. normal horse (roan) serum, in 32 days.	235	0.2 c. c. normal horse (roan) serum into brain. 2 hours latter bled for serum.	Slight symptoms.
1189A	5 c. c. serum G. P. 1189 subcutaneously.	2	0.15 c. c. normal horse (Teddy) serum into brain.	Marked symptoms.
1190	11 injections, each 5 c. c. normal horse (roan) serum, in 35 days.	232	0.2 c. c. normal horse (roan) serum into brain. 2 hours later bled for serum.	Mild symptoms.
1190A	9 c. c. serum G. P. 1190 subcutaneously.	2	0.15 c. c. normal horse (Teddy) serum into brain.	Slight symptoms.
1191	12 injections, each 5 c. c. normal horse (roan) serum, in 39 days.	228	0.2 c. c. normal horse (roan) serum into brain. 2 hours later bled for serum.	Slight symptoms.
1191A	9 c. c. serum G. P. 1191 subcutaneously.	2	0.15 c. c. normal horse (Teddy) serum into brain.	Marked symptoms.

It is therefore plain that anaphylactin does exist in the blood serum of immune guinea pigs.

We add the following examples showing that the presence of anaphylactin may not always be demonstrated. In these experiments the blood serum was collected in one of two ways. From guinea pigs Nos. 1000 to 1007, inclusive, the blood was drawn from the heart, allowed to clot at room temperature, and the serum pipetted off next morning. In guinea pigs Nos. 1138 to 1147, inclusive, the blood was drawn from the large vessels of the neck, defibrinated by whipping, centrifugated, and the clear supernatant serum used to inject into the normal guinea pig.

Perhaps the long interval may account for the negative results.

TABLE No. 36.—*Anaphylactin.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1000	4 c. c. normal guinea pig serum subcutaneously.	26	6 c. c. normal horse (roan) serum intraperitoneally.	No symptoms.
1001	1.5 c. c. serum G. P. 8038, which had been subcutaneously inoculated 49 days prior with 0.24 c. c. toxine No.9+1/540 c. c. antitoxic horse serum (NY 13C).	26	.....do	No symptoms.
1002	2 c.c. serum G.P. 8048, which had been subcutaneously inoculated 49 days prior with 0.24 c. c. toxine No. 9+1/360 c. c. antitoxic horse serum (Welc.).	26	.....do	No symptoms.
1003	3.5 c. c. same serum	26	.....do	No symptoms.
1004	4 c. c. serum G. P. 8051, which had been subcutaneously inoculated 49 days prior with 0.24 c. c. toxine No. 9 + 1/400 antitoxic horse serum (Welc.).	26	.....do	No symptoms.
1005	4 c. c. serum G. P. 8045, which had been subcutaneously inoculated 49 days prior with 0.24 c. c. toxine No. 9 + 1/560 c. c. antitoxic horse serum (Welc.).	26	.....do	No symptoms.
1006	4 c. c. same serum	26	.....do	No symptoms.
1007	6 c. c. serum G. P. 8050, which had been subcutaneously inoculated 49 days prior with 0.24 c. c. toxine No. 9 + 1/320 c. c. antitoxic horse serum (Welc.).	26	.....do	No symptoms.
1138	1.5 c. c. serum G. P. 8128, which had received 27 subcutaneous injections normal horse serum in a period of 65 days and bled 21 days after last injection.	16	0.25 c. c. normal horse (roan) serum into brain.	No symptoms.
1139	3.5 c. c. same serum	16	5 c. c. normal horse serum intraperitoneally.	No symptoms.
1140	1.5 c. c. serum G. P. 8127, which had received 27 subcutaneous injections normal horse serum in a period of 65 days and bled 21 days after last injection.	19	0.25 c. c. normal horse serum into brain.	Mild symptoms.

TABLE NO. 36.—*Anaphylactin*—Continued.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1141	4 c. c. same serum	19	5 c. c. normal horse serum intraperitoneally.	Slight symptoms.
1142	1.5 c. c. serum G. P. 8003, which had been subcutaneously injected with 0.24 c. c. toxine No. 9 + 1/420 c. c. antitoxic horse serum (NY 1013) and bled 99 days afterwards.	19	0.25 c. c. normal horse serum into brain.	Mild symptoms.
1143	20 c. c. same serum	19	5 c. c. normal horse serum intraperitoneally.	Slight symptoms.
1144	1.5 c. c. serum G. P. 8126, which had received 27 subcutaneous injections normal horse serum in a period of 65 days and bled 21 days after last injection.	19	0.25 c. c. normal horse serum into brain.	Mild symptoms.
1145	3 c. c. same serum	19	5 c. c. normal horse serum intraperitoneally.	No symptoms.
1146	4 c. c. serum G. P. 7846, which had been subcutaneously injected with 0.24 c. c. toxine No. 9 + 1/310 c. c. antitoxic horse serum (Alex. spl. 192) and bled 139 days afterwards.	19	0.25 c. c. normal horse serum into brain.	Very severe symptoms.
1147	1.5 c. c. same serum	19	do	Severe symptoms.

TABLE NO. 37.—*Anaphylactin*—*Miscellaneous.*

G.P. No.	First injection.	Interval in days.	Second injection.	Result.
696T	0.0006 c. c. tetanus toxine A + 0.0025 c. c. antitoxic horse serum (PI).	27	2 c. c. antitoxic horse serum (Natl. IX) intraperitoneally.	Symptoms (?).
			Next day 0.2 c. c. normal horse (roan) serum into brain.	No symptoms.
695T	0.0006 c. c. tetanus toxine A + 0.002 c. c. antitoxic horse serum (PI).	27	2 c. c. antitoxic horse serum (Natl. IX) intraperitoneally.	Symptoms (?).
			Next day 0.2 c. c. normal horse (roan) serum into brain.	No symptoms.
711T	0.0006 c. c. tetanus toxine A + 0.0075 c. c. antitoxic horse serum (Standard T2).	27	2 c. c. antitoxic horse serum (Natl. IX) intraperitoneally.	Symptoms (?).
			Next day 0.2 c. c. normal horse (roan) serum into brain.	No symptoms.
	Half an hour later above 3 pigs bled; blood mixed, defibrinated, and 12 c. c. of serum obtained.			
1392	12 c. c. blood serum of above 3 guinea pigs intraperitoneally.	2	0.2 c. c. normal horse (Frank) serum into brain.	No symptoms.

PART 6.—MECHANISM.

LESIONS.

Gay and Southard,[a] 1907, found in guinea pigs dying from a second injection of serum, and in those which had severe symptoms and were later chloroformed, lesions which are interpreted as explaining the mechanism of anaphylaxis. They state that "the study of the histopathology of this serum disease shows us that we have to deal with an intricate cell reaction demonstrable by definite cell lesions." Considerable hemorrhages, rather definitely localized, are the characteristic gross lesions. The hemorrhages may be in one or several organs, gastric hemorrhages being especially frequent. Microscopically there are, in addition to the naked-eye hemorrhages, minute interstitial and oozing hemorrhages. They also found fttay changes in voluntary muscle fiber, heart muscle fiber, and in nerve fiber.

That the conjestion and dilatation of the blood vessels found in the abdominal cavity and the hemorrhages upon the mucosa of the stomach are not characteristic of death due to anaphylaxis is evident from the fact that we have found that in violent death produced by large subcutaneous injections of chloral cyanhydrin or hydrocyanic acid there are somewhat similar congestions and hemorrhages.[b] Further, we have lately had the opportunity to examine a guinea pig whose death was caused by suffocation in an atmosphere of carbon dioxid. In the stomach and lungs of this guinea pig lesions were found that, so far as the congestion and hemorrhages are concerned, were somewhat similar to those described in guinea pigs dying from a second injection of horse serum.

We were especially struck by the fact that the macroscopic congestions and hemorrhages may be absent in guinea pigs poisoned by intracerebral injections.

Further, this congestion and dilatation of the vessels of the abdominal cavity is well known to occur in shock and other states.

We were also unable to confirm Gay and Southard's findings in regard to the fatty changes.

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[a] Journ. Med. Research, May, 1907, p. 143.

[b] Rosenau, M. J., and Anderson, John F.: A stomach lesion in guinea pigs caused by diphtheria toxine and its bearing on experimental gastric ulcer. Journ. Infec. Dis., vol. 4, No. 1, Jan., 1907, p. 1–7.

We studied a large number of pigs in which death occurred within thirty minutes of the second injection of serum; also, a moderate number which were killed by chloroform or otherwise from one to four hours after the second injection.

We are indebted to our colleague Dr. W. W. Miller, United States Public Health and Marine-Hospital Service, for the following studies upon the post-mortem appearances and pathology of the tissues of guinea pigs dead of anaphylaxis.

The most noticeable lesion to the naked eye is the marked dilatation of the small veins and capillaries of the body, but most noticeable in those of the abdominal viscera. Associated with this in about 25 per cent of the cases are hemorrhages in the mucosa of the stomach and, more rarely, of the intestines. Minute hemorrhages 1 mm. in size are occasionally observed on the surface of the lungs; no hemorrhages of the heart muscle, spleen, pericardium, or striped muscle, as described by Gay and Southard, have been seen.

For microscopic study the tissues from 16 guinea pigs were utilized. These pigs had received the second dose of serum in three ways, viz, by subcutaneous, intraperitoneal, and intracranial injection. Material was selected from all the viscera and from the striped muscles and nervous systems. Particular attention was given to the study of tissues for the fatty changes described by Gay and Southard. For this purpose sections made with the freezing microtome were used to a great extent, as it is generally recognized that fresh material is superior to that prepared and sectioned in the customary way in celloidin and paraffin, although it must be admitted, as Gay and Southard contend, that such preparations are not as permanent or suitable for micro-photographic purposes. For a general study of microscopic changes tissues were fixed in 10 per cent formalin, in formalin and alcohol (5 and 85 per cent), and Zenker.

For fatty degeneration, fresh tissue and tissue fixed for twenty-four hours in 5 to 10 per cent watery solution of formalin was used and sectioned with the freezing microtome; for nerve tissue, formalin 5 to 10 per cent, Orth's fluid, and Müller's fluid.

As a stain for general purposes, hematoxylin and eosin were used.

For staining fat, Marchi's method was carried out as follows: Fixation in 10 per cent formalin and Müller's fluid six to ten days, in Marchi's mixture six to ten days; kept throughout in the dark; then washed twenty-three hours in running water; hardened in alcohol and ether celloidin as quickly as possible. Clove oil celloidin was not used, as it causes general blackening.

For frozen sections of tissue fixed in 10 per cent formalin for twenty-four hours, the admirable method so highly recommended by Schmörl was used, viz, sections placed in Marchi fluid in closed vessels in the paraffin oven for one-half to one hour, washed quickly in water, and mounted in glycerin or dehydrated over night in alcohol and mounted in balsam.

As controls in this work, tissue was used from normal pigs, from a pig dead of puerperal sepsis with marked fatty changes in liver and kidney, and from pigs killed with diphtheria toxine. Sati's method of osmization was also used with some of the specimens.

Fresh and formalinized tissue was stained by the admirable method of Torrain Smith (nile blue sulphate); also, Sudan III.

Results.—In sections stained for general study the sole difference from normal tissue consists in the marked dilatation of the veins and capillaries, especially of the stomach and intestines, accompanied by extravasation of blood at points where the vessels are ruptured. The thin-walled veins of the mucosa of the stomach are often greatly distended. In the few instances where a ruptured point in the vessel was seen in section the extravasation of blood was from the portion of the vein nearest the inner surface of the mucosa. The veins of the submucosa participated in the dilatation, but were not so markedly enlarged as the veins and capillaries of the mucosa. No evidence of general giving way of the capillaries with extravasation of blood into the tissues was noted.

The small veins and capillaries of the intestines, kidney, heart, and muscles were found distended, but not nearly to the extent found in the stomach wall. No hemorrhages were observed in the heart walls or in the liver and striped muscles.

As regards fatty changes in the lining endothelium of the blood vessels, in the gastric mucosa or striped muscles, none was observed, although carefully sought for. The focal fatty changes described by Gay and Southard were not found. Neither were the "nodal" changes in peripheral nerves made out.

We find, then, that congestion and sometimes hemorrhage take place in guinea pigs dead of anaphylaxis, but these lesions are not specific. We were unable to demonstrate the fatty lesions and know, further, that they occur in other states. We are therefore unable to confirm the observations of Gay and Southard along these lines, and believe that these changes do not explain the mechanism of anaphylaxis.

PART 7.—RELATION OF TOXIC ACTION UPON GUINEA PIGS TO SERUM THERAPY.

THE RELATION OF SERUM ANAPHYLAXIS IN THE GUINEA PIG TO SERUM THERAPY.

Besredka and Steinhart[a] were the first to point out that the second injection may be given into the brain of guinea pigs. When a small quantity of horse serum is injected into the brain of a sensitized guinea pig the symptoms appear promptly and often with great violence, and death is a common result.

Besredka[b] believes that intracerebral injections may be used as a measure for the toxicity of therapeutic serums. He states that, measured in this way, different serums show a wide gamut of toxicity, the fatal dose varying from $\frac{1}{4}$ to $\frac{1}{128}$ c. c. He believes that this toxicity resides in the serum and not in the cellular elements; further, that the serums of horses living under apparently the same conditions have about the same toxicity, individual variations being rare and of little importance. He concludes that, in a general way, all serums that incite in guinea pigs grave anaphylactic phenomena in doses of $\frac{1}{16}$ to $\frac{1}{20}$ c. c. and, *a priori*, above this amount should be considered toxic.

We doubt whether there is a relation between the toxicity of serums as tested upon guinea pigs in this way and their power to produce the serum disease or collapse or sudden death in man. The unfortunate accidents, such as collapse and occasional death, depend more upon the sensitization of the individual than upon the so-called toxicity of the serum used.

Fortunately we were able to obtain two antidiphtheric serums which had been used in two cases of sudden death.

Case No. 1.—Serum No. 2277. Reported by Dr. S. N. Wiley, Norristown, Pa., Journ. Am. Med. Assn., vol. 50, Jan. 11, 1908, p. 137. Mr. E. W., aged 34 years, splendid physique, best of health. Prophylactic injection of 1,000 units antidiphtheric serum. Site of inoculation 4 inches above Poupart's ligament. Within two minutes had violent symptoms—anxious expression, itching, burning, labored breathing; lips, face, and neck swollen and red; paralysis; convulsions. Died within five minutes of injection.

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[a] Besredka and Steinhart, Ann. de l'Inst. Pasteur, 1907, vol. 21, p. 117.

[b] Ann. de l'Inst. Pasteur, 1907, vol. 21, p. 777.

The toxicity of this serum, i. e., another package of the same laboratory number, was tested upon the following series of guinea pigs:

Table No. 38.—*Toxicity of serum No. 2277.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9147	0.23 c.c. toxine No. 7+1/420 c.c. antitoxic horse serum (PD. 09913).	97	0.1 c. c. antitoxic horse serum (2277) into brain.	Dead in 7 minutes.
9131	0.23 c.c. toxine No. 7+1/250 c.c. antitoxic horse serum (Natl. V. 24).	97	.....do...........................	Dead in 8 minutes.
9136	0.23 c.c. toxine No. 7+1/300 c.c. antitoxic horse serum (Natl. V. 24.)	97	0.05 c. c. antitoxic horse serum (2277) into brain.	Dead in 35 minutes.
9151	0.23 c.c. toxine No. 7+1/380 c.c. antitoxic horse serum (PD. 09491).	97	.....do...........................	Very severe symptoms.
9161	0.23 c.c. toxine No. 7+1/320 c.c. antitoxic horse serum (Mem. C20).	67	2 c. c. antitoxic horse serum (2277) intraperitoneally.	Dead in 3 hours.
9170	0.23 c.c. toxine No. 7+1/330 c.c. antitoxic horse serum (Mul. 2438).	67	2 c. c. antitoxic horse serum (2277) subcutaneously.	Very severe symptoms.

Case No. 2.—Serum No. 2295. Reported by Dr. H. F. Gillette, Cuba, N. Y., Journ. Am. Med. Assn., vol. 50, Jan. 4, 1908, p. 40. Mr. B., 52 years old. Had asthma and bronchial catarrh. Urine and heart normal. Rheumatic attack fifteen years prior. Coughed and raised plenty of sputum. Injection of 2,000 units antitoxic serum under left scapula. Prickling sensation in chest and neck; labored breathing; pulse regular and full. Seized with tonic spasm. Died within five minutes after injection.

The serum, i. e., another package of the same laboratory number, used in this case was tested for toxicity upon the following series of guinea pigs:

TABLE No. 39.—*Toxicity of serum No. 2295.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9135	0.23 c. c. toxine No. 7+1/370 c. c. antitoxic horse serum (Natl. V.24).	97	0.1 c. c. antitoxic horse serum (2295) into brain.	Dead in 20 minutes.
9133	0.23 c. c. toxine No. 7+1/370 c. c. antitoxic horse serum (Natl. V.24).	97	0.05 c. c. antitoxic horse serum (2295) into brain.	Dead in 22 minutes.
9132	0.23 c. c. toxine No. 7+1/310 c. c. antitoxic horse serum (Natl. V.24).	97	.....do........................	Dead in 11 minutes.
9373 m	0.23 c. c. toxine No. 7+1/1080 c. c. antitoxic horse serum (NY. 306).	97	.....do........................	Severe symptoms.
9171	0.23 c. c. toxine No. 7+1/410 c. c. antitoxic horse serum (Mul. 2438).	67	1 c. c. antitoxic horse serum (2295) subcutaneously.	Dead in 90 minutes.
9164	0.23 c. c. toxine No. 7+1/400 c. c. antitoxic horse serum (Mem. C23).	67	1 c. c. antitoxic horse serum (2295) intraperitoneally.	Very severe symptoms.

It has interested us very much to find that these two cases, and also others that have come to our notice, were in asthmatics. In our first publication we suggested that the essential lesion of serum anaphylaxis is probably localized in the respiratory center, and the association of asthma and hypersusceptibility to horse serum in man would seem to lend weight to this hypothesis. The knowledge of the fact that the injection of horse serum into some asthmatics may be attended with danger should be considered in the use of antitoxin.

In order to determine the comparative toxicity of the above two serums (Nos. 2277 and 2295); we submit the following experiments showing the toxicity of serums which have been largely used in human therapy without untoward effects.

The following serums (Nos. 2364, 2369, and 2442) were kindly sent us by Dr. A. P. Hitchens, who states "he has had no report whatever concerning any untoward effect resulting from the use of either of these numbers:"

No. 2364 is antitoxic serum.

No. 2369 is antitoxic globulin.

No. 2442 is antitoxic serum.

TABLE NO. 40.—*Comparative toxicity of other serums (Nos. 2442, 2369, and 2364.)*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9936	0.23 c. c. toxine No. 7+1/260 c. c. antitoxic horse serum (Alex. 192).	47	0.05 c. c. antitoxic horse serum (Mul. 2442) into brain.	Dead in 4 minutes.
		47	.....do.....	Dead in 5 minutes.
9943	0.23 c. c. toxine No. 7+1/290 c. c. antitoxic horse serum (Alex. 192.)	47	0.1 c. c. antitoxic horse serum (Mul. 2442) into brain.	Dead in 3 minutes.
9937	0.23 c. c. toxine No. 7+1/320 c. c. antitoxic horse serum (Alex. 192).			
9941	0.23 c. c. toxine No. 7+1/300 c. c. antitoxic horse serum (Alex. 192).	47	0.05 c. c. antitoxic horse serum (Mul. 2369) into brain.	Dead in 8 minutes.
9939	0.23 c. c. toxine No. 7+1/310 c. c. antitoxic horse serum (Alex. 192).	47	.....do.....	Dead in 5 minutes.
9933	0.23 c. c. toxine No. 7+1/270 c. c. antitoxic horse serum (Alex. 192).	47	0.1 c. c. antitoxic horse serum (Mul. 2442) into brain.	Dead in 3 minutes.
9945	0.23 c. c. toxine No. 7+1/180 c. c. antitoxic horse serum (Alex. 192).	47	0.05. c. c. antitoxic horse serum (Mul. 2364) into brain.	Marked symptoms
9946	.....do.....	47	.....do.....	Dead in 12 minutes
9934	0.23 c. c. toxine No. 7+1/270 c. c. antitoxic horse serum (Alex. 192).	47	0.1 c. c. antitoxic horse serum (Mul. 2364) into brain.	Dead in 3 minutes.
9942	0.23 c. c. toxine No. 7+1/300 c. c. antitoxic horse serum (Alex. 192).	47	.....do.....	Marked symptoms

The following diphtheria antitoxic horse serum (123A) was kindly sent us by Dr. William H. Park, who reports that it is of moderate strength and has given very good results in the hospital.

TABLE NO. 41.—*Comparative toxicity of serum No. 123A.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9245	0.23 c. c. toxine No. 7+1/480 c. c. antitoxic horse serum (Ldrl. 11) subcutaneously.	131	0.05 c. c. antitoxic horse serum (Park 123A) into brain.	Very severe symptoms.
9251	0.23 c. c. toxine No. 7+1/1000 c. c. antitoxic horse serum (Ldrl. 212) subcutaneously.	131	.....do.....	Very severe symptoms.
9242	0.23 c. c. toxine No. 7+1/360 c. c. antitoxic horse serum (Mul. 2377) subcutaneously.	131	.....do.....	Very severe symptoms.
8759	0.245 c. c. toxine No. 42+1/320 c. c. antitoxic horse serum (Strn. 1429) subcutaneously.	232	0.1 c. c. antitoxic horse serum (Park 123A) into brain.	Very severe symptoms.
9253	0.23 c. c. toxine No. 7+1/1500 c. c. antitoxic horse serum (Ldrl. 21B) subcutaneously.	131	.....do.....	Dead in 10 minutes.
9239	0.23 c. c. toxine No. 7+1/800 c. c. antitoxic horse serum (Ldrl. 57) subcutaneously.	131	0.2 c. c. antitoxic horse serum (Park 123A) into brain.	Dead in 5 minutes.

The following two serums were kindly furnished by Dr. E. M. Houghton, and were extensively used in human therapy, but no complaints were received concerning them:

Table No. 42.—*Comparative toxicity of serum No. 08725C.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9635	0.23 c. c. toxine No. 7+1/600 c. c. antitoxic horse serum (Alex. A249) subcutaneously.	87	0.05 c. c. antitoxic horse serum (PD 08725C) into the brain.	Very severe symptoms.
9644	0.23 c. c. toxine No. 7+1/680 c. c. antitoxic horse serum (Alex. A249) subcutaneously.	87	.....do........................	Very severe symptoms.
9645	0.23 c. c. toxine No. 7+1/660 c. c. antitoxic horse serum (Alex. A249) subcutaneously.	87	.....do........................	Dead in 4 minutes.
9632	0.23 c. c. toxine No. 7+1/640 c. c. antitoxic horse serum (Alex. A249) subcutaneously.	87	0.05 c. c. antitoxic horse serum (PD 09043C) into the brain.	Dead in 8 minutes.
9646	0.23 c. c. toxine No. 7+1/660 c.c. antitoxic horse serum (Alex. A249) subcutaneously.	87	.....do........................	Dead in 4 minutes.
9648	0.23 c. c. toxine No. 7+1/640 c c. antitoxic horse serum (Alex. A249) subcutaneously.	87	.....do........................	Dead in 15 minutes

As a control, the following serums—some of them French antidiphtheric and some normal horse serums—are given for comparison:

Table No. 43.—*Comparative toxicity of French and other serums.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
532	0.0006 c. c. tetanus toxine A+0.23 c. c. antitoxic horse serum (Ehrlich standard).	65	0.25 c. c. normal horse (roan) serum into brain.	Dead in 5 minutes.
8497	0.24 c. c. toxine No. 42+1/200 c. c. antitoxic horse serum (Cutter 1828).	18	.....do........................	Very severe symptoms.
8359	0.24 c. c. toxine No. 9+1/1260 c. c. antitoxic horse serum (N. Y. 305).	35	.....do........................	Dead in 5 minutes.
9550	0.23 c. c. toxine No. 7+1/240 c. c. antitoxic horse serum (PD 07635).	62	0.2 c. c. antistreptococcic serum (Past. Inst.) into brain.	Dead in 3 minutes.
9557	0.23 c. c. toxine No. 7+1/280 c. c. antitoxic horse serum (Welc. 2L839).	62	.....do........................	Marked symptoms.
9572	0.23 c. c. toxine No. 7+1/260 c. c. antitoxic horse serum (Alex. A245).	62	.....do........................	Dead in 4 minutes.
9580	0.23 c. c. toxine No. 7+1/300 c. c. antitoxic horse serum (Alex. A245).	65	0.2 c. c. antitoxic horse serum (Lyons) into brain.	Dead in 3 minutes.

Table No. 43.—*Comparative toxicity of French and other serums*—Continued.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9552	0.23 c. c. toxine No. 7+1/500 c. c. antitoxic horse serum (Ldrl. 58A).	65	0.2 c. c. antitoxic horse serum (Lyons) into brain.	Dead in 3 minutes.
9590	0.23 c. c. toxine No. 7+1/320 c. c. antitoxic horse serum (Alex. A245).	65	.....do........................	Dead in 4 minutes.

It therefore seems plain that the serums which do not produce untoward symptoms when injected into man, are quite as toxic upon sensitized guinea pigs, as the serums which have been followed by serious symptoms when injected into man. We believe the difference lies in the susceptibility of the individual and not in the toxicity of the serum.

PART 8.—THE EFFECT OF REPEATED INJECTIONS.

THE IMMUNIZING ACTION OF REPEATED INJECTIONS.

We found it desirable to obtain further data upon the effects of repeated injections of horse serum into guinea pigs in order to answer the question whether guinea pigs may be definitely immunized by repeated injections.

It will be seen by the following table that 10 injections of 2 c. c. of serum subcutaneously was not sufficient to render guinea pigs entirely immune. These four guinea pigs all showed mild symptoms when tested intraperitoneally fifteen or seventeen days after the last injection.

Reference to Tables No. 34, 35, 36, pages 36, 37, 38, shows the same results.

TABLE NO. 44.—*The immunizing action of repeated injections.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1371	10 injections, each 2 c. c., normal horse (roan) serum, subcutaneously, covering a period of 17 days.	15	6 c. c. normal horse (roan) serum intraperitoneally.	Mild symptoms.
1369	.....do	17	.....do	Mild symptoms.
1372	.....do	17	.....do	Mild symptoms.
1374	.....do	17	.....do	Mild symptoms.

THE SENSITIZING ACTION OF REPEATED INJECTIONS OF SMALL AMOUNTS.

The following table shows that the repeated injections of small amounts are apparently as potent in sensitizing guinea pigs as one small injection.

TABLE NO. 45.—*Sensitizing action of repeated injections of small amounts.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1364	5 injections, each 0.001 c. c., normal horse (roan) serum, subcutaneously, covering a period of 8 days.	[a] 23	6 c. c. normal horse (roan) serum intraperitoneally.	Dead in 40 minutes.
1365	.....do	23	6 c. c. normal horse (roan) serum subcutaneously.	Dead in 70 minutes.
1366	.....do	23	.....do	Dead in 75 minutes.
1367	.....do	23	0.05 c. c. normal horse (roan) serum into brain.	Severe symptoms.
1368	.....do	23	0.2 c. c. normal horse (roan) serum into brain.	Dead in 5 minutes.
1363	.....do	23	0.05 c. c. normal horse (roan) serum into brain.	Severe symptoms.

[a] From last injection.

THE EFFECT OF REPEATED SMALL INJECTIONS UPON SENSITIVE GUINEA PIGS.

We were interested in determining whether repeated small injections would immunize sensitive guinea pigs without the production of anaphylactic symptoms. For this purpose we injected a series of guinea pigs subcutaneously with normal serum in amounts too small to produce apparent symptoms and repeated the injections daily for twenty days. One series received 0.001 c. c. subcutaneously (total 0.02 c. c.) the other series 0.01 c. c. subcutaneously (total 0.2 c. c.). It will be seen from the following table that these repeated injections had little, if any, effect upon the susceptibility of these guinea pigs.

TABLE NO. 46.—*Effect of repeated small injections upon sensitive guinea pigs.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9716	0.23 c.c. toxine No. 7+1/600 c. c. antitoxic horse serum (Schr. II). 48 days later 20 subcutaneous injections, each 0.001 c. c., normal horse (Frank) serum daily.	[a]2	0.2 c. c. normal horse (Teddy) serum into brain.	Very severe symptoms.
9673	0.23 c. c. toxine No. 7+1/560 c. c. antitoxic horse serum (PD 08022). 48 days later 20 injections 0.001 c. c. daily.	2	.....do.........................	Marked symptoms.
9678	0.23 c.c. toxine No. 7+1/520 c. c. antitoxic horse serum (PD 08022). 48 days later 20 injections 0.001 c. c. daily.	2	.....do.........................	Marked symptoms.
9714	0.23 c. c. toxine No. 7+1/500 c. c. antitoxic horse serum (Schr. II). 48 days later 20 injections 0.01 c. c. daily.	2	.....do.........................	Dead in 5 minutes.
9715	.....do.........................	2	.....do.........................	Marked symptoms.
9677	0.23 c. c. toxine No. 7+1/520 c. c. antitoxic horse serum (PD 08022). 48 days later 20 injections 0.01 c. c. daily.	2	.....do.........................	Marked symptoms.
9155	0.23 c. c. toxine No. 7+1/200 c. c. antitoxic horse serum (Cutter 1856). 60 days later 10 injections 0.001 c. c. each, covering period of 20 days.	11	6 c. c. normal horse (roan) serum intraperitoneally.	Dead in 120 minutes.
9156	0.23 c. c. toxine No. 7+1/240 c. c. antitoxic horse serum (Cutter 1856). 60 days later 10 injections 0.001 c. c. each, covering period of 20 days.	11	0.2 c. c. normal horse (roan) serum into brain.	Dead in 7 minutes.
9252	0.23 c. c. toxine No. 7+1/400 c. c. antitoxic horse serum (Schr. II). 60 days later 10 injections 0.001 c. c. each, covering period of 20 days.	11	6 c. c. normal horse (roan) serum subcutaneously.	Dead in 90 minutes.

[a] After last injection.

TABLE No. 46.—*Effect of repeated small injections upon sensitive guinea pigs*—Con.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9186	0.23 c. c. toxine No. 7+1/270 c. c. antitoxic horse serum (Mul. 2362). 60 days later 10 injections 0.001 c. c. each, covering period of 20 days.	11	0.05 c. c. normal horse (roan) serum into brain.	Severe symptoms.
9157	0.23 c. c. toxine No. 7+1/290 c. c. antitoxic horse serum (Cutter 1856). 60 days later 10 injections 0.001 c. c. each, covering period of 20 days.	11	6 c. c. normal horse (roan) serum subcutaneously.	Slight symptoms.

ATTEMPTS TO IMMUNIZE SENSITIVE GUINEA PIGS BY REPEATED INJECTIONS OF HEATED SERUM.

In the following series sensitized guinea pigs were injected with 1 c. c. of normal horse serum heated to 100° C. for one hour. The serum was first diluted in the proportion of 1 part to 3 of water to prevent coagulation. The 1 c. c. of the mixture injected into the guinea pigs represents, therefore, 0.25 c. c. of horse serum. The pigs received from 20 to 25 injections subcutaneously. They therefore received a total amount of 5 to 6 c. c. of normal horse serum. It is evident from the table that these injections failed to materially modify the susceptibility of the guinea pigs.

TABLE No. 47.—*Effect of heated serum upon sensitive guinea pigs.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
9197	0.23 c. c. toxine No. 7+1/400 c. c. antitoxic horse serum (Schr. II). 62 days later 20 injections 1 c. c. daily normal horse (roan) serum heated 100° C. 1 hour, subcutaneously.	[a]10	0.2 c. c. normal horse (roan) serum into brain.	Dead in 10 minutes
9179	0.23 c. c. toxine No. 7+1/540 c. c. antitoxic horse serum (Mul. 2443). 62 days later 22 injections 1 c. c. daily heated serum.	8	6 c. c. normal horse (roan) serum intraperitoneally.	Severe symptoms.
9180	0.23 c. c. toxine No. 7+1/680 c. c. antitoxic horse serum (Mul. 2443). 62 days later 25 injections 1 c. c. daily heated serum.	4	6 c. c. normal horse (roan) serum subcutaneously.	Marked symptoms.
9191	0.23 c. c. toxine No. 7+1/400 c. c. antitoxic horse serum (Mul. 2358). 62 days later 24 injections 1 c. c. daily heated serum.	6	.....do..........................	Very severe symptoms.

[a] After last injection.

PART 9.—THE RELATION OF ANAPHYLAXIS TO THE TOXEMIAS OF PREGNANCY.

The symptoms which cause puerperal eclampsia and the conditions under which it occurs suggest that anaphylaxis may explain some of the mystery of this state.

It occurred to us that either the blood or proteid substances in solution from the fetus or the placenta may first sensitize the mother. A subsequent introduction into the system of the mother of a similar substance may explain the convulsions and the symptoms which occur in a certain class of the toxemias of pregnancy.

"Through the establishment of the pathological anatomy of the condition a general agreement has been reached that puerperal eclampsia must be included among the diseases caused by toxic materials of unknown origin and nature."[a] A certain class of the toxemias of pregnancy are sometimes spoken of as reflex or neurotic origin.

There seems to be a fair agreement that the placenta must be the source of toxic material, especially as typical cases of eclampsia and pernicious vomiting have been observed in patients with hydatid mole, in which cases, of course, toxic matter of fetal origin could be eliminated. Furthermore, eclampsia may appear after the fetus has been removed. Much attention was therefore given to the hypothesis elaborated about four years ago by Veit, Weichardt, and others that through the entrance of placental cells into the circulation of the mother an intoxication was caused either by disintegration of the cells and the formation of toxic substances or in the development of antisubstances by the maternal organism.

In spite of much experimentation and discussion, however, no satisfactory conclusions have yet been reached concerning the validity of this hypothesis, and Martin has secured some very valuable evidence that at least in rabbits entrance of their own placental elements into the circulation in large amounts does not cause any serious disturbance. So far as we are aware, we are the first to suggest that certain of the toxemias of pregnancy may be a condition of hypersusceptibility.

Along these lines we first made a number of experiments to determine whether the fetal blood of the guinea pig could sensitize the mother guinea pig. We injected a number of female guinea pigs, both pregnant and not pregnant, with fetal blood, and after an appropriate interval gave them a second injection of the same material.

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[a] From a recent discussion of the theories concerning the causes of the toxemias of pregnancy. Editorial in the Journal of the American Medical Association, vol. 50, No. 2, Jan. 11, 1908, p. 124.

All these experiments resulted negatively, which was anticipated from our previous studies upon the effect of homologous blood serums. This tends to confirm the clinical observations that the poisons causing the toxemias of pregnancy do not come from the fetus.

We then made a series of experiments upon female guinea pigs with placental extracts. The placenta (almost at full term) was ground up in a mortar and allowed to "autolyze" about an hour at room temperature, and some of the resulting extract was injected subcutaneously into female guinea pigs.

After an interval of twenty-two days the guinea pigs were again inoculated with a placental extract. This time the placenta was allowed to "autolyze" three hours in the incubator (37° C.). Five pigs were tested with this placental extract; three of them were given 6 c. c. into the peritoneum, two of these three showing pronounced symptoms of anaphylaxis. The remaining one showed slight symptoms. Six cubic centimeters of the same placental extract injected into the peritoneal cavity of two young normal guinea pigs as a control produced no apparent effect. The remaining pigs were injected with small quantities of the extract intracerebrally, with negative results.

These experiments were repeated with precisely similar results. Thus four more guinea pigs were sensitized with different quantities of guinea-pig placental extract, and after a period of twenty-two days were given a second injection of similar placental extract. The extract used at both the first and second injections in these four pigs was autolyzed three hours in the incubator, then strained through gauze. All four of them showed definite symptoms.

In a third experiment three guinea pigs were sensitized subcutaneously with placental extract (three hours at 37°), and after an interval of twenty-four days the second injection was given directly into the circulation by the intracardiac method. These three pigs showed severe and early symptoms, including convulsions. Three normal control guinea pigs treated in the same manner did not respond. The three pigs that responded to the second intracardiac injection were autopsied and found to have a fresh current-jelly clot in the pericardial sac. How much this hemorrhage, which is probably due to the puncture of superficial vessels of the heart, may account for the symptoms is doubtful. Further work along this line is in progress.

From this limited series it is evident that the mother guinea pig may be sensitized with the autolytic products of her own placenta. These experiments naturally suggest that there may be a certain relation between some cases of puerperal eclampsia and the phenomenon in the guinea pig which we are studying. Further studies along this line are now being made, especially to determine whether the liver lesions may thus be produced in the guinea pig and other animals.

PART 10.—MISCELLANY.

TIME.

The longest time that has elapsed between the first and the second injections in a guinea pig in our experiments has been two years two days (732 days). It therefore seems that guinea pigs once sensitized with mixtures of antitoxic horse serum and diphtheria toxine are sensitive throughout practically the remainder of their lives.

Time.

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
4523	0.19 c.c. toxine No. 7+1 immunity unit antitoxic horse serum (B27).	732	6 c. c. normal horse (roan) serum intraperitoneally.	Dead in 60 minutes.

Table No. 48.—*Clam juice.*

G. P. No.	First injection.	Interval in days.	Second injection.	Result.
1650	0.1 c. c. clam extract subcutaneously.	127	6 c. c. clam extract subcutaneously.	Marked symptoms.
1651	.....do	127	.....do	Do.
1652	.....do	127	6 c. c. clam extract intraperitoneally.	Dead in 50 minutes.
1653	.....do	127	.....do	Marked symptoms.
1654	.....do	127	.....do	Do.

The above series shows that the protein matter of clams may sensitize and poison guinea pigs.

PART II.—SUMMARY AND CONCLUSIONS.

The *period of incubation* of serum anaphylaxis is about seven days in guinea pigs sensitized in the brain and about nine days in guinea pigs sensitized subcutaneously. It also appears that the sensitization comes on somewhat gradually.

Judged by our results and the work of others, the period of incubation is quite constant.

It seems that the period of incubation is not appreciably prolonged by a large sensitizing dose.

Animals sensitized with horse serum alone remain so for a long period of time (at least 245 days). Guinea pigs sensitized with the toxine-antitoxin mixture remain sensitive throughout the remainder of their life (at least 732 days).

The *sensitizing principle* is gradually influenced by heat. It disappears almost entirely when horse serum is heated to 100° C. for one hour.

Guinea pigs may be sensitized by intracerebral injections, provided quantities of 0.000,1 c. c. or more are used. We obtained negative results with sensitizing doses of 0.000,01 c. c. into the brain.

Guinea pigs may be sensitized by dropping horse serum upon the eye.

The *toxic principle* in horse serum is gradually destroyed by heat.

A temperature of 70° C. for one hour does not seem appreciably to diminish the poisonous property of horse serum, but it seems to be affected at 80° C. for one hour. At 90° C. for one hour it still remains slightly toxic, but at 100° C. for one hour the toxicity apparently disappears.

The difference in the effect of heat upon the sensitizing and the toxic principle may be more apparent than real, for exceedingly minute amounts of serum will sensitize guinea pigs, while it would take a very large quantity of weakened serum to produce symptoms at the second injection.

The toxicity of horse serum does not appear to diminish with the age of the serum.

No favorable influence upon the anaphylactic state was obtained by injecting pancreatin, potassium oxalate, pepsin, sodium sulphate, magnesium sulphate, peptone, calcium chloride, and calcium acetate into guinea pigs the day before they were tested.

Iodine also apparently had no modifying effect upon serum anaphylaxis, whether dissolved in the serum or injected separately into the guinea pig.

Methemaglobin-producing substances, such as the nitrites, do not hinder anaphylaxis.

Ether narcosis masks the symptoms, but does not prevent the fatal issue of a second injection.

Further attempts to find free antibodies to neutralize the toxic action of horse serum by treating it with the sensitized guinea pig serum, and also with the brain substance of sensitized guinea pigs, proved negative.

The *specific nature of anaphylaxis* is further shown by various experiments. For example, guinea pigs sensitized with three separate proteins, viz, horse serum, egg white, and cow's milk, contain three separate anaphylactins in their blood.

Guinea pigs sensitized with human milk do not react to a second injection of cow's milk. This again indicates not only the specific nature of the anaphylactic reaction, but suggests differences between the protein matter of human and cow's milk.

Guinea pigs sensitized with sheep's milk react to a subsequent injection of cow's milk.

Guinea pigs sensitized with dog's milk do not react to a subsequent injection of cow's milk.

Guinea pigs sensitized with hen egg white react to a subsequent injection of duck egg white; and guinea pigs sensitized with duck egg white react to a subsequent injection of hen egg white. The results of similar studies with goose, guinea hen, crane, pigeon, and turkey egg albumin are recorded in the text.

The anaphylactic reaction in the guinea pig, therefore, seems to be specific in the sense that the precipitins are specific. That is, there is a group reaction in the proteins of allied species, but no reaction between the proteins of widely different species or between proteins of widely different origin.

A substance known as "*anaphylactin*" is present in the blood serum of sensitized guinea pigs. This substance is not present during the period of incubation.

We have been unable to demonstrate the presence of anaphylactin in the blood serum of man, the monkey, and the cat.

Anaphylactin is present in the blood serum of immune guinea pigs.

The mechanism of anaphylaxis.—We find that congestion and sometimes hemorrhages may be present in guinea pigs dead of anaphylaxis, but these lesions are not always apparent, and, furthermore, are not specific.

We were unable to demonstrate fatty lesions in guinea pigs dead of anaphylaxis, and know, further, they occur in other states.

We believe that these morphological alterations do not explain the mechanism of anaphylaxis. It is probable that the mechanism will not be unraveled until further light is shed upon the chemistry of protein metabolism.

Cases of sudden death in man.—Our experiments demonstrate that the horse serum used in cases followed by sudden death is no more toxic for guinea pigs than antitoxic horse serums used extensively in human therapy without untoward symptoms.

It is our belief that it is not the special toxicity of the horse serum, but the sensitization of the patient, which accounts for the collapse or sudden death sometimes following the injection of horse serum.

We are still unable to account for the ways in which man may be sensitized to a foreign protein. It seems perfectly plain, however, that man may be so sensitized.

In previous publications we suggested that the essential lesion of serum anaphylaxis is probably localized in the respiratory center, and the association of asthma and hypersusceptibility to horse serum in man seems to lend some weight to this hypothesis.

The knowledge of the fact that an injection of horse serum into some asthmatics may be attended with danger should be considered in the use of antitoxin.

The *repeated injections* of small amounts of horse serum sensitizes guinea pigs.

Repeated injections of large amounts render guinea pigs partially immune.

Repeated injections of small amounts of serum into sensitized guinea pigs have no appreciable effect.

Sensitized guinea pigs can not be immunized by repeated injections of heated serum (100° C. for one hour).

We suggest a possible relation between the *toxemias of pregnancy and anaphylaxis.*

Guinea pigs can not be sensitized with guinea-pig fetal blood. This shows that the fetal blood of the guinea pig does not contain an alien protein for the mother.

Guinea pigs may be sensitized and subsequently poisoned with guinea-pig placental extracts.

LIST OF HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH AND MARINE-HOSPITAL SERVICE.

The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, located in Washington, was authorized by act of Congress, March 3, 1901.

The following *bulletins* [Bulls. Nos. 1–7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.] have been issued:

*No. 1.—Preliminary note on the viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau.

*No. 3.—Sulphur dioxid as a germicidal agent. By H. D. Geddings.

*No. 4.—Viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 5.—An investigation of a pathogenic microbe (*B. typhi murium* Danyz) applied to the destruction of rats. By M. J. Rosenau.

*No. 6.—Disinfection against mosquitoes with formaldehyd and sulphur dioxid. By M. J. Rosenau.

No. 7.—Laboratory technique: Ring test for indol, by S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis; Microphotography with simple apparatus, by H. B. Parker.

By act of Congress approved July 1, 1902, the name of the "United States Marine-Hospital Service" was changed to the "Public Health and Marine-Hospital Service of the United States," and three new divisions were added to the Hygienic Laboratory.

Since the change of name of the Service the bulletins of the Hygienic Laboratory have been continued in the same numerical order, as follows:

*No. 8.—Laboratory course in pathology and bacteriology. By M. J. Rosenau. (Revised edition, March, 1904.)

*No. 9.—Presence of tetanus in commercial gelatin. By John F. Anderson.

No. 10.—Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States. By Ch. Wardell Stiles.

*No 11.—An experimental investigation of *Trypanosoma lewisi*. By Edward Francis.

*No. 12.—The bacteriological impurities of vaccine virus; an experimental study. By M. J. Rosenau.

*No. 13.—A statistical study of the intestinal parasites of 500 white male patients at the United Staes Government Hospital for the Insane; by Philip E. Garrison, Brayton H. Ransom, and Earle C. Stevenson. A parasitic roundworm (*Agamomermis culicis* n. g., n. sp.) in American mosquitoes (*Culex sollicitans*); by Ch. Wardell Stiles. The type species of the cestode genus *Hymenolepis;* by Ch. Wardell Stiles.

No. 14.—Spotted fever (tick fever) of the Rocky Mountains; a new disease. By John F. Anderson.

No. 15.—Inefficiency of ferrous sulphate as an antiseptic and germicide. By Allan J. McLaughlin.

*No. 16.—The antiseptic and germicidal properties of glycerin. By M. J. Rosenau.

*No. 17.—Illustrated key to the trematode parasites of man. By Ch. Wardell Stiles.

*No. 18.—An account of the tapeworms of the genus *Hymenolepis* parasitic in man, including reports of several new cases of the dwarf tapeworm (*H. nana*) in the United States. By Brayton H. Ransom.

*No. 19.—A method for inoculating animals with precise amounts. By M. J. Rosenau.

*No. 20.—A zoological investigation into the cause, transmission, and source of Rocky Mountain "spotted fever." By Ch. Wardell Stiles.

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TREASURY DEPARTMENT
Public Health and Marine-Hospital Service of the United States

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HYGIENIC LABORATORY.—BULLETIN No. 46
June, 1908

HEPATOZOON PERNICIOSUM (N. G., N. SP.); A HAEMOGREGARINE PATHOGENIC FOR WHITE RATS; WITH A DESCRIPTION OF THE SEXUAL CYCLE IN THE INTERMEDIATE HOST, A MITE (*LELAPS ECHIDNINUS*)

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SUMMARY.

Hepatozoon perniciosum, n. g., n. sp., is a hæmogregarine found in white rats in Washington, D. C., and the cause of an epizootic observed among these animals. The protozoon is conveyed by a mite (*Lelaps echidninus* Berlese), which is the true intermediate host. Infection is transmitted to the rat when the mite is swallowed by the rat.

The mites are ecto-parasites upon the rats, from which they receive infection by sucking the blood.

Multiplication of the hæmogregarine in the rat takes place in the liver. In the stomach of the mite the hæmogregarines conjugate and form an ookinet, which penetrates the stomach wall and completes its development in the body tissues of the mite.

The illustrations in this bulletin have been prepared from drawings made by the laboratory artist, Prof. Leonard H. Wilder.

HEPATOZOON PERNICIOSUM (N. G., N. SP.)—A HÆMOGREGARINE PATHOGENIC FOR WHITE RATS; WITH A DESCRIPTION OF THE SEXUAL CYCLE IN THE INTERMEDIATE HOST, A MITE (LELAPS ECHIDNINUS BERLESE).[a]

By William Whitfield Miller,
Assistant Surgeon, U. S. Public Health and Marine-Hospital Service, Washington, D. C.

INTRODUCTION.

Although the first of the Hæmocytozoa to be discovered (*Lankesterella ranarum*, by Lankester in 1871) was a hæmogregarine, our knowledge to-day of this important group is far from complete. A great number of hæmatozoa have been described in cold-blooded animals, in nearly all of which there is a more or less close resemblance to the gregarine type. The most noteworthy peculiarity of these parasites is the wormlike form assumed during at least some portion of the life cycle. They are, at some period, endoglobular; but they lack the ameboid motion, and pigmentation so characteristic of the hæmamœbæ. Multiplication occurs by division into two or into many individuals.

Classifying them according to the vertebrate host, the following is a brief description of the known species.

HÆMOGREGARINIDÆ OF AMPHIBIA.

About ten species of hæmogregarines in batrachians are known, all of which affect the red blood cells of frogs and toads. Until quite recently the belief was unquestioned that sexual as well as nonsexual multiplication occurred in the same host. In fact, Hintze[b] interpreted certain appearances as a conjugation of the parasites and the formation of an ookinet which entered the intestinal epithelium, became encysted, was cast off in the feces and served, when ingested by another frog, to propagate the parasite.

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[a] Manuscript submitted for publication June 25, 1908.

[b] Hintze, R.: Lebensweise und Entwicklung von *Lankesterella minima* (Chaussat). Zoolog. Jahrb., Abt. f. Anat. u. Ontog., Bd. 15, Heft 4, 1904, pp. 693–730.

Lühe believes this observation to be in error and regards the oocyst as coccidial in nature. The multiple infections of protozoa (flagellates, coccidia, and hæmogregarines) found in frogs, often in the same animal, renders it very difficult to determine positively to which parasite certain phases in the life cycle belong.

A recent observation by Durham[a] upon the life history of *Drepanidium* in a South American toad is of special interest. He found that nearly all the toads were infested with a species of tick. In the stomach of the tick were seen forms of the *Drepanidium* apparently in copulation, also peculiar cysts 60 micra in diameter, which he believed to be some stage in the life cycle of the parasite. Durham regards the tick as the probable intermediate host, although this is not definitely established.

The studies of Billet[b] and also some recent observations of Laveran and Nègre[c] seem to indicate that trypanosomes may play a part in the life cycle of *Lankesterella* and other hæmogregarines. Beyond question, a great deal of work is necessary upon this group before many doubtful points can be clarified.

HÆMOGREGARINIDÆ OF SNAKES AND LIZARDS.

Lühe[d] mentions nineteen species affecting the red blood corpuscles of snakes and lizards. He divides them into two genera, *Karyolysus* and *Hæmogregarina*. A noteworthy peculiarity of the former is the existence of large and small trophozoites (without intermediate stages)—the macro-merozoites and micro-merozoites. Schizogony takes place in red blood cells in the capillaries of internal organs. The membrane remaining after destruction of the red cell forms a sort of a cyst wall. The schizonts are of two types, macro-schizonts and micro-schizonts, corresponding to the two varieties of merozoites.

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[a] Durham, H. E.: *Drepandium* in the toad. Liverpool School of Tropical Medicine, Memoir VII—Report of the yellow-fever expedition to Para, Liverpool, 1902, pp. 78–79.

[b] Billet, A.: Sur le *Trypanosoma inopinatum* de la grenouille verte de l'Algérie et sa relation possible avec les *Drepanidium*. C. R. Soc. Biol., Paris, vol. 57, No. 27, 1904, pp. 161–165.

[c] Laveran, A., & Nègre: Sur un protozoaire parasite de *Hyalomma ægyptium*. C. R. Soc. Biol., Paris, vol. 58, no. 21, 1905, pp. 964–966.

[d] Lühe, M.: In Handbuch der Tropenkrankheiten, C. Mense, Leipzig, 1906, Bd. III, pp. 209–210.

Lutz[a] regards the small forms as male and the large as female. He has never seen evidence of conjugation of the trophozoites in the blood of the host. The intermediate host is unknown.

HÆMOGRÆGARINIDÆ OF TURTLES AND CROCODILES.

A large number of hæmogregarines have been described in turtles. *Hæmogregarina stepanovi* Danielewsky has been carefully studied by Danielewsky, and in its sexual development by Siegel.[b]

In *Hæmogregarina stepanovi* the gametocytes, according to Siegel, are the elongated trophozoites which are bent into a U shape on account of their length being greater than that of the red blood cell which they inhabit. Schizogony occurs as in other hæmogregarines. There is no dimorphism of the schizonts. The invertebrate host is a leech, in the gut of which the gametocytes develop and fertilization takes place. The ookinet which develops from the zygote invades the intestinal wall and enters the esophageal glands, where elongated sporozoites are formed. Infection of the turtle, to which the leech next attaches itself, is said to take place in a manner similar to the malarial infection from mosquitoes. Siegel believes that the ovary and eggs of the leech may be invaded by the sporozoites, and the next generation of leeches thus infected.

HÆMOGREGARINIDÆ OF FISHES.

About twelve species are known. *Hæmogregarina bigemina*, from the blood of blennies (*Blennius pholis*), has been described by Laveran and Mesnil.[c] In the nonsexual multiplication of this parasite by binary fission a resemblance to the manner of multiplication in piroplasma is observed, and incidentally to that in trypanosomes. In this connection it is interesting to note that trypanosomes have been found very frequently in the blood of fishes, which also harbor hæmogregarines. The group is as yet imperfectly known. The mode of transmission of the infection has not been determined.

In all hæmogregarines of cold-blooded animals which have been studied, nonsexual multiplication or schizogony takes place in the red blood corpuscles, usually in some internal organ. The merozoites, when set free, invade other red blood cells, and increase considerably in size before reaching maturity.

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[a] Lutz, A.: Ueber die *Drepanidien* der Schlangen. Ein Beitrag zur Kenntniss des Hamosporidien. Centblt. f. Bakt., 1. Abt., vol. 29, 1901, pp. 390–398.

[b] Siegel: Die geschlechtliche Entwicklung von *Hæmogregarina stepanovi* in Rüsselegel *Placobdella catenigera*. Arch. f. Protistenkde., 1903, Bd. 2, H. 3, pp. 339–342.

[c] Laveran, A., & Mesnil, F.: Deux hémogrégarines nouvelles des poissons. C. R. de l'Acad. des Sci., vol. 133, 1901, pp. 572–577.

HÆMOGREGARINIDÆ OF MAMMALS.

Although the first protozoan blood parasites to be observed, with the exception of trypanosomes, were hæmogregarines, and since that time a great number have been discovered in cold-blooded animals and more or less completely described, knowledge of such forms in warm-blooded animals dates back only three or four years.

In 1905 James[a] published a description of a new parasite found in the white blood cells of pariah dogs in India, their occurrence having been previously noted by Bentley.[b] James did not regard the parasite, which he named *Leucocytozoon canis*, as a hæmogregarine, but considered its relationship to parasites in the blood of birds, certain forms of which appear to infect the white blood corpuscles.

These so-called "leucocytozoa" were first described by Danielewsky[c] in the blood of owls.

Quite recently Schaudinn,[d] Laveran,[e] and Berestneff[f] have shown that the forms of this parasite (now known as *Leucocytozoon ziemanni*), which resemble parasites in leucocytes, are in reality macrogametes to which are attached portions of the nucleus of the red cell originally invaded. The name is somewhat misleading.

James found *Leucocytozoon canis* present in the blood of 6 out of 45 dogs examined. The polymorphonuclear cells were the only variety affected. The largest percentage of infected corpuscles recorded was 9 per cent. In one single instance only was a parasite found free in a stained blood smear. On one occasion a free form was seen in a fresh preparation, having escaped from the leucocyte by reason of its continuous vermicular movements. The wormlike body remained in contact with the host cell, but continued its movements for an hour or more.

The intracorpuscular forms, when stained by Romanowsky's method, are described as oblong bean-shaped bodies enveloped by the protoplasm of the leucocyte and apparently surrounded by a delicate cyst. In addition to the bean-shaped bodies, James describes parasites, almost round in shape, with a very indistinct capsule. The leucocytes affected are of the polymorphonuclear variety. The nucleus in 86 per cent of the parasites is situated near one extremity and is oval or round in shape, and stains like the nuclei of the leucocytes.

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[a] James: On a parasite found in the white corpuscles of the blood of dogs. Sci. Mem Officers Med. & San. Dept. Govt. India, 1905, n. s., no. 14.

[b] Bentley: Preliminary note upon a leucocytozoon of the dog. Brit. med. journ., May 6, 1905, p. 988.

[c] Danielewsky, B.: La parasitologie comparée du sang. Kharkoff, 1889. Also. Developpement des parasites malariques dans les leucocytes des oiseaux. Ann. de l'Inst. Pasteur, 1890, p. 427.

[d] Schaudinn, F.: Generations- und Wirtswechsel bei *Trypanosoma* und *Spirochæte*. Arb. a. d. Kaiserl. Gesundheitsamte, vol. 20, 1904, p. 387–439.

[e] Laveran, A.: Contribution à l'étude de *Hæmamœba ziemanni*. C. R. Soc. Biol., vol. 55, 1903, p. 620–623.

[f] Berestneff, N.: Ueber das Leucocytozoön Danielewsky. Arch. f. Protistenkde, vol. 3, 1904, p. 376–386.

The manner in which nonsexual multiplication takes place in the bone marrow is described by Christophers.[a]

Since the discovery of *Leucocytozoon canis* by Bentley a number of somewhat similar parasites have been observed in the blood of mammals, all in tropical regions. In some instances they affect only the white corpuscles, in others only the red cells, but never both. Certain peculiarities and their similarity to hæmogregarines in cold-blooded animals are sufficient to identify them in the Hæmogregarinidæ.

In 1905 Balfour[b] described a new parasite in the red blood cells of *Jaculus jaculus*, the jerboa, or kangaroo rat, of Sudan. This organism, according to Laveran, is a hæmogregarine.

The trophozoite, which is either free or lies in the remains of a red blood cell, is sausage shaped, nonpigmented, and nonmotile. It measures 5.5 to 7 micra in length and from 1.4 to 2.8 micra in breadth. When stained by the Leishmann-Romanowsky method, the nucleus is large, oval, and centrally located. In the peripheral blood all forms are in about the same stage of development. The animal host does not suffer in health.

The parasite (*Haemogregarina balfouri*) of the jerboa is bent slightly upon itself and its length is a little greater than that of the red corpuscles. In addition to the form described, Balfour observed on one occasion a free motile vermicule form without flagellum. In a stained specimen which showed this stage the total length was 15.5 micra, one extremity being finely tapered to a point, the other rounded and containing chromatin granules. Multiplication occurs by schizogony. The schizonts are found in the liver, kidney, and bone marrow, in the form of cysts. The cyst walls appear to be formed of the remnant of the cell of the organ invaded. In the liver the schizonts appear quite similar to those of certain coccidia. The manner of invasion of the liver cells has not been determined. No sexual cycle or mode of transmission is known. Balfour states that no naked-eye indications of disease are present in an infected animal. The spleen is not enlarged. He also mentions the fact that he has discovered what seems to be the same parasite (*H. balfouri*) in the mononuclear leucocytes of a rat (*Mus decumanus*) in Khartoum.

In 1905 Christophers[c] discovered a parasite in the red blood cells of the Indian field rat (*Gerbillus indicus*) which resembles *H. balfouri.* The affected corpuscles (somewhat enlarged) contained a minute motionless vermicule with a short, sharply flexed tail (trophozoite). A distinct space surrounded the parasite. In some fresh preparations there were free parasites similar to the above, except that the pointed extremity was no longer bent. They exhibited active gliding movements, also twisting and turning motions. In stained specimens the trophozoites were in the red cells and appeared to be enveloped by a capsule. A large chromatin body was situated in the "tail" of the parasite, also occasional scattered granules.

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[a] Christophers, S. R.: *Leucocytozoon canis*. Sci. Mem. Officers Med. & San. Dept. Govt. India, 1906, n. s., no. 26.

[b] Balfour, A.: A hæmogregarine of mammals. Journ. Trop. Med., vol. 8, 1905, p. 241; vol. 9, 1906, pp. 81–84.

[c] Christophers, S. R.: *Hæmogregarina gerbilli*. Sci. Mem. Officers Med. & San. Dept. Gov. India, 1905, n. s., no. 18.

No certain sexual differentiation of the trophozoites could be made out. Although all the organs of infected rats were carefully studied, no evidence of nonsexual multiplication could be found. Infected rats showed no constitutional disturbances. Differential leucocyte counts indicated that the percentage of different kinds of leucocytes differed but little from the normal.

The only ecto-parasites found upon the rats were insects—a new species of louse (*Hæmatopinus*) measuring 1.4 by .5 mm. Certain structures were encountered in the dissection of these lice which Christophers regarded as phases of the sexual cycle of *H. gerbilli.* Although experiments were made to infect lice from infected animals, no recorded attempts were made to transmit the infection from one animal to another by means of lice. In his experiments a number of lice were placed upon a young heavily infected rat. These were removed at stated intervals and examined. On the fourth day two lice were removed and examined. In the midgut and intestine vermicules in active motion were observed. On the seventh day large cysts were observed lying free in the body cavity of the insect. They were of large size, measuring as much as 350 micra in diameter. When dissected out they appeared as minute white spheres which, with the microscope, were observed to be filled with minute cysts containing six or eight crescentic bodies. Younger cysts measuring 10 micra in diameter were also seen, also intermediate ones varying in size between the smallest and the largest. No satisfactory stained specimens of these intermediate stages were obtained. No abnormal appearance was observed in the salivary glands of any of the lice examined. When ruptured cysts were mixed with the blood of the Indian rat and examined with the microscope, the crescents became active vermicules.

No forms suggesting sexual elements or conjugation were observed by Christophers. In a recent work this writer[a] states, regarding the life cycle of *H. gerbilli* in *Hæmatopinus stephensi:* "Vermicules are freely liberated in the midgut and in the body cavity large cysts are found; but, as a result of work on *L. canis* in the tick, I am doubtful whether the cysts have anything to do with the hæmogregarine."

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[a] Christophers, S. R.: The sexual cycle of *Leucocytozoon canis* in the tick. Sci. Mem. Officers Med. & San. Dept. Govt. India, 1907, no. 28.

Patton [a] has described a parasite in the large mononuclear leucocytes of palm squirrels. Forms measuring 10 micra in length were seen, one extremity being bent and somewhat pointed, resembling a ail. Free vermicules exhibited slow "streaming" movements. Paton made a number of leucocyte counts upon blood from infected and noninfected squirrels and found both a relative and numerical ncrease of the large mononuclears in the former. Smears were made from all the organs of the squirrel, but no evidence of multiplication was observed.

Lice (a species of *Hæmatopinus*) were found upon many of the squirrels examined. Upon dissection active vermicules measuring 15.5 micra were seen in the gut of the lice which had been placed upon infected squirrels. No further development was observed, nor were any parasitic bodies found in the ovaries or salivary glands.

Christophers [b] has recently published his observations upon the sexual cycle of *Leucocytozoon canis* in the tick. He had previously described the schizogony, which takes place in the bone marrow, but never in the liver or kidney. The merozoites are formed in cysts. One cyst may contain 30 or more merozoites.

The sexual cycle in the dog tick (*Rhipicephalus sanguineus*) is described as follows: In the gut of ticks examined immediately after removal from infected dogs free vermicules are seen; after twenty-four hours they have penetrated the cells of the gut wall and are seen n various stages of fission, which precedes the formation of the sexual elements. On the third day conjugation bodies and the first stages of he development of the fertilized oocyst are seen. Mature oocysts are observed on the fourth day. According to Christophers, the vermicules, after entering the intestinal cell, divide into two, four, or even eight vermicules. Two of these vermicules then conjugate and an oocyst is formed, which, when fully developed, measures 14 micra n diameter and splits up into 12 to 14 sporozoites, without an investing capsule. The entire process takes place in the epithelium of the gut wall and not in the cœlomic cavity of the tick.

From the description of Christophers it seems difficult to understand his interpretation of some of the stages observed. Especially t would seem difficult to distinguish the parasites formed by fission of the vermicules from the free sporozoites. He mentions no feature which distinguishes the male elements (microgametes) from the female (macrogametes).

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[a] Patton, W. S.: On a parasite found in the white corpuscles of the blood of palm squirrels. Sci. Mem. Officers Med. & San. Dept. Govt. India, 1906, n. s., no. 24.

[b] Christophers, S. R.: The sexual cycle of *Leucocytozoon canis* in the tick. Sci. Mem. Officers Med. & San. Dept. Govt. India, 1907, no. 28.

The method of inoculation of a dog with *L. canis* by the tick is unknown. Christophers states that since the female tick does not suck blood again after repletion, the passage of the sporozoite into the ova seems a necessity if the parasite is to regain entrance into the mammalian host. He has been unable to find sporozoites in the ova or, in fact, anywhere except in the gut of the ticks.

Adie,[a] in India, has observed in blood smears from *Mus rattus*, in each instance accompanied by trypanosomes, a leucocytozoon with an oval nucleus and without cytocyst, and about one-third the length of the leucocyte in which it was inclosed. Further details regarding it are lacking.

Summary. A critical examination of the literature dealing with the Hæmogregarinidæ reveals the fact that almost nothing is definitely known concerning their sexual cycle and manner of transmission.

HEPATOZOON PERNICIOSUM.

Material and Technique.

An examination of a blood smear from a white rat which had died from an unknown cause revealed a large number of protozoan parasites somewhat resembling *Leucocytozoon canis*. It was found upon inquiry that the stock of white rats kept for laboratory use, in cages, in a wooden building adjoining the stable was being greatly depleted by disease. Many of the animals became anæmic and emaciated and after a longer or shorter period, usually a week or more, died. Upon examination it was found that some of the cages were infested with mites, which were present in large numbers upon the rats. Some of these mites, taken from an infected rat, were found to harbor the leucocytic parasite found in the blood of the rats. A more extended study revealed the interesting fact that the sexual phase of the parasite occurs in the mite.

Seventeen rats, four of which died later, were made the basis of a study of the parasite in the rat. In addition, a large amount of material was obtained from rats experimentally infected. Material in a perfectly fresh condition was taken from all organs and preserved in fixing fluids. All the customary fixatives were employed, but the most satisfactory reagent for preserving the parasites in tissues was found to be formalin (formalin = 40 per cent formaldehyde). In strengths of 4 to 10 per cent of formalin in normal salt solution the tissues were immersed for twenty-four hours, then washed out in water and preserved in 70 to 80 per cent alcohol. Sections varying in thickness from 5 to 15 micra were made from paraffin-embedded tissue. The stains used for sections were Weigert's iron hematoxylin, Delafield's hematoxylin, and Giemsa's solution for Romanowsky staining. Smears from blood and the various organs were prepared, both dry and fixed in alcohol, formol alcohol, and methyl alcohol. Giemsa's and Wright's stains were found the most satisfactory for blood smears.

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[a] Adie, J. R.: Note on a leucocytozoon found in *Mus rattus* in the Punjaub. Journ. trop. med., vol. 9, 1906, p. 325

In drawing blood from living rats some difficulty is usually experienced. If a portion of the tail (about 1½ inches) is removed, sufficient blood for smears is readily obtained. If the blood examination is to be repeated daily, a slight manipulation causes the granulation tissue formed upon the stump to bleed freely. The increased number of leucocytes on account of the slight inflammatory reaction from the wound does not interfere with the detection of the parasite. Obviously blood obtained in this manner could not be used for differential or total counts of the white blood cells, in which instance it is necessary to remove another small piece from the extremity.

In handling the rats a pair of long curved surgical dressing forceps, with which the animal is grasped by the loose skin at the back of the neck, are very convenient.

Hepatozoon perniciosum in the Blood of White Rats.

When stained smears made from the blood of infected rats are examined with the microscope, large oval nucleated bodies are seen embedded in the protoplasm of leucocytes. The white cells affected are almost invariably large mononuclear lymphocytes, seldom transitional leucocytes, and very rarely polymorphonuclear leucocytes. The mononuclear and transitional cells of the rat's blood resemble very closely cells of the same type in human blood. The nuclei stain strongly with basic stains, and the protoplasm is free of basic staining granules. The nuclei of the former variety are almost always excentrically located in the cell protoplasm, and the parasite, when present, is usually situated beside the nucleus, where the protoplasm is most abundant. In transitional cells the parasite is often wedged in the angle formed by the bending of the nucleus. The white cells which contain parasites appear to suffer in no way by reason of their presence. Evidence of degenerative or other changes is lacking.

A marked increase in the number of leucocytes, especially of the large mononuclear variety, is invariably observed when parasites are present. This increase varies in direct proportion to the number of parasites in the blood. The blood in severe infections, shortly before death, strongly suggests that seen in human lymphatic leukemia, both in the fresh and stained condition. Nucleated red cells, which are present in small numbers in normal rat's blood, are often greatly increased in numbers. A series of actual and differential leucocyte counts of the blood of rats, with infections of varying severity, is given below.

Rat No.	Large lymphocytes containing parasites.	Number of parasites free.	Total white blood cells.	Large mononuclears.	Transitional.	Polymorphonuclears.	Eosinophiles.	Small mononuclears.
	Per cent.			*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
1	72.	2	36,000	62.	6.2	20.9	1.1	9.8
2	16	4	32,600	48	5.5	31.1	2.1	8.3
3	53.5	6	102,000	65.1	9.6	18.3	.8	7.2
4	30	0	16,000	51.3	4.5	31.2	.6	12.4
5	82.5	6	128,000	78.7	6.5	11.6	.3	2.9
6	39	0	28,500	45.8	6	39.6	.5	8.1
7	48	1	64,000	50.1	8.6	35.5	1.8	4
8	2	0	12,600	30.2	8.5	51	2	8.3
9	12	0	10,800	35.6	10.5	48	1.5	4.4
10	20.5	0	15,200	30.5	7	50.7	1.6	10.2

A number of red blood cell counts made upon the blood of heavily infected rats gave from 1,900,000 to 3,200,000 per cubic millimeter. Hemoglobin, with the Tallquist scale, from 20 to 60 per cent.

PARASITES IN FRESH BLOOD.

When a drop of blood is spread out thinly between cover slip and slide the parasites are readily made out in the leucocytes. They appear as large rod-shaped bodies with rounded extremities and are slightly more refractile to light than the leucocytes. No nucleus can be detected. In the fresh condition the parasite appears longer and more slender than when stained. It is often longer than the leucocyte in which it is contained, the protoplasm of the latter appearing to be stretched somewhat in order to encompass the parasite.

The first studies made upon *H. perniciosum* in the blood were limited to material obtained from naturally infected rats, in all of which the infection had existed for some time before the discovery of the microorganism. In the blood of these rats nearly all the parasites were in leucocytes, and very few were observed free. In rats artificially infected, and in which daily blood examinations were made from the beginning, conditions were found to be somewhat different at the inception of the disease.

The first parasites observed in recently infected animals were free in the blood plasma, few in number, and actively motile. Later, the common encysted forms appeared and the motile forms—the "vermicules"—disappeared. In a few rats, vermicules were found during the course of the disease and especially just before death. The vermicules, as observed, are slender bodies 15 to 16 micra in length, with clear delicately striated nongranular protoplasm, somewhat refractile to light. The anterior extremity, bluntly pointed, is

lways the one directed forward when in motion. The posterior
xtremity is narrower, rounded, and nearer the nucleus. The move-
ients, size, and appearance of the vermicules are identical with
iose of vermicules formed from sporozoites, which will be described
ter in detail.

PARASITES STAINED.

In blood smears stained by Giemsa's or Wright's stain the parasites
ppear as elongated oval bodies, broader and shorter than in the fresh
lood, measuring on the average 12 by 6 micra. The nucleus stains
lmost the same color as the nucleus of the leucocyte in which it ap-
ears. The protoplasm of the parasite stains pale blue (more strongly
t the periphery), is uniform in character, and is free of granules. The
ucleus usually occupies the middle third of the parasite; occasion-
lly it is located at the extremity. In outline the common form is
omewhat quadrilateral with rounded corners or is irregularly oval.
nstead of being made up of chromatin granules the nucleus is com-
osed of skeins or bands of chromatin, often arranged in a more or
ss parallel fashion at right angles to the parasite. It is probable
at the nucleus is quite thin and flat, since parasites are frequently
en in which the chromatin is arranged in a single narrow band
ear and parallel to one side. Surrounding each parasite and separat-
g it from the protoplasm of the leucocyte is a delicate cyst wall,
hich appears usually as a clear, unstained, narrow area of even
ickness. The superficial layer of protoplasm of the parasite
ems condensed. That the cyst is an integral part of the latter
nd not of the leucocyte is shown by the fact that free encysted
rms are occasionally seen.

The behavior of the parasites toward stains is an additional evi-
ence of the presence of a cyst wall. When less powerful stains are
nployed than those mentioned the parasite stains with great
ifficulty, although the leucocytes are strongly stained. In faintly
ained specimens the parasites appear as colorless, refractile bodies,
e interior being unstained.

It is noteworthy that in *H. perniciosum*, as in other hæmogre-
arines of mammals, the forms met with in the circulation—the tro-
hozoites—are approximately equal in size. Moreover, the dimen-
ons do not vary greatly in different animals or in the same animal
different times. This peculiarity is explained, as will be shown
ter, by the fact that the parasites are discharged only when fully
rown into the circulating blood.

In stained specimens the free vermicules are slender, curved or
raight, with blue staining protoplasm, devoid of granules and
ithout a capsule. The nucleus is formed of several rounded chro-
atic bodies (pl. II). One extremity is often pointed, and the

vermicule is longer and slenderer than the encysted parasite. The latter unquestionably originates from the vermicule and all gradations between the two are observed, especially in smears from the liver.

In the first stage the vermicule becomes broader and shorter, retaining the granular nucleus; next, the latter is changed to the bandlike or homogeneous form, either before or after being engulfed by a phagocytic mononuclear or transitional white cell; and finally a capsule is developed. The ultimate fate of the parasites is uncertain. In rats with a mild grade of infection the encysted trophozoites may be found in moderate numbers six to eight weeks or even longer after the onset of the disease; yet when the livers of such animals are examined no schizonts are found. From this it is evident that the trophozoites after becoming encysted may continue in the circulation, acting merely as foreign bodies, for some time after their formation. The pathogenic effects appear, as in other hæmatozoa, to be caused by toxic substances set free during the stage of multiplication.

In addition to the forms described, others are sometimes observed. These consist of slight variations from the usual motionless type, the trophozoite. Large oval or even spherical parasites are seen, with banded nuclei, sometimes free, more often in leucocytes, and rarely showing signs of a cyst wall (pl. II). Such parasites are common in liver smears, but rare in the circulating blood. No evidence of sexual differentiation of the trophozoites has been observed, unless the forms just described be regarded as sexually different from the usual type. It seems more probable that these large parasites are very young schizonts, which occasionally escape from the liver after partial development, into the blood stream.

NONSEXUAL MULTIPLICATION OF *H. PERNICIOSUM* IN THE RAT.

It has already been mentioned that the trophozoites found in the large lymphocytes are practically uniform in size and appearance. No form is seen which may be construed as young or partly developed. The explanation of this lies in the fact that multiplication and development into mature parasites takes place in the cells of a single organ, the liver. At an early stage of infection, when parasites are numerous in the blood, a section of the liver shows many parasites in various stages of schizogony. Similar forms have been found in no other organ, although carefully sought for. The trophozoites are especially abundant in the spleen, kidney, and brain, but there is no evidence of multiplication in these organs.

In the capillaries of the liver are many large lymphocytes containing encysted parasites similar to those encountered in the peripheral circulation. In addition, there are parasites whose contour blends with the protoplasm of the leucocyte and appears to be unprovided with a cyst (pl. II). Free parasites, some of which are large and oval in shape (pl. II), are also abundant.

The multiplication of the parasite takes place in a liver cell. The earliest stage is a small spherical form 10 micra in diameter, with a large vesicular nucleus composed of chromatin granules in a fine mesh work, and a large karyosome. It is embedded in the protoplasm of the liver cell, the nucleus of the latter being pushed to one side. The cavity formed in the liver cell is fully occupied by the parasite. As development proceeds the parasite increases in size and the nucleus divides into two. This stage is known as schizogony and the parasite becomes a schizont. Many small, round granules are found in the latter when viewed in the fresh condition. The two nuclei divide and subdivide until from 12 to 20 daughter nuclei are formed. The schizont increases in size and the liver cell becomes a mere shell, the nucleus of which, after undergoing the changes characteristic of degeneration, finally disappears. A delicate cyst wall is formed around the developing schizont, which, at maturity, is ovoid and measures, on the average, 25 by 30 micra. Some, however, are larger and may measure 28 by 35 micra.

The nuclei of the schizont are arranged at the two extremities, as shown in longitudinal sections (pl. III). In fresh cysts studied at this stage the central mass of protoplasm is granular, with a clear area at the ends. At a later stage short rods of clear protoplasm, each containing a nucleus, are seen at the poles. These rods increase in length, the proximal extremities growing toward one another; the central granular mass diminishes in size. Eventually the clear rod-shaped bodies have become fully grown merozoites and occupy the entire cyst except for a small granular "rest" body. When the merozoites are fully grown, the cyst containing them becomes soft and gelatinous; later it ruptures and the merozoites escape, becoming free moving vermicules. The number of merozoites is from 12 to 20; the average 16.

In scrapings made from the cut surface of a liver in which the schizonts and cysts are numerous, their structure and the ease with which they are ruptured is readily determined. In sections from tissue, fixed and embedded in the usual method, the contents of the cyst shrink to a greater degree than the liver tissue and artificial spaces are formed around the schizonts (pl. III, fig. 1).

In heavily infected livers the number of cysts is very large. Their presence is associated with extensive fatty changes in the liver parenchyma. When a small piece of fresh liver tissue is spread out thinly between slide and cover slip and examined with the microscope on a warm stage, the contents of the ripe cysts are seen to be in motion. The merozoites move slowly about, gliding over one another, like a mass of worms. Often such a cyst is seen to rupture and the merozoites escape. In this event they move about as free vermicules, bending and twisting and gliding about exactly as will be described later in the case of vermicules formed from sporozoites, from which they can not be distinguished. Some of these vermicules enter the liver capillaries and later the circulating blood; others enter fresh liver cells and repeat the process of multiplication.

In stained smears made from the liver the various stages in the transformation of the free vermicule into the encysted trophozoite are readily traced (pl. II). In the former the nucleus is composed of large chromatin granules and the parasite is slender and elongated. It then becomes contracted in length and oval in shape; still later the nucleus is disposed in the characteristic parallel skein arrangement, and finally a cyst develops, the parasite being engulfed by a large mononuclear lymphocyte. No evidence of sexual dimorphism of the schizonts has been noted. The merozoites, when released from the cysts, are fully developed and do not subsequently increase in size in the blood. The large mononuclear leucocytes are found in in unusual numbers in the liver, in the sinuses of the lymphatic glands, the thymus, spleen, and bone marrow.

There seems to be little doubt that the vermicules, which are taken in by leucocytes in the liver and elsewhere and become encapsulated trophozoites, undergo no further development in the rat. Those vermicules which escape the phagocytic action of these cells and enter other liver cells repeat the process of schizogony. The majority perhaps are carried into the peripheral circulation in the leucocytes.

The liver cysts and merozoites are almost exactly duplicated in size and appearance by the sporocysts and sporozoites in the body of the intermediate host, to be described later. Encysted trophozoites are especially abundant in the spleen, lungs, kidneys, and brain, in the blood vessels, and lymph spaces.

CLINICAL SYMPTOMS IN WHITE RATS.

When the infection is slight, the rat often presents no evidence of disease. For a month or so the blood may show only a few parasites encysted in the leucocytes. This type of the disease is more common in old rats.

Frequently the disease begins gradually, with a few parasites in the blood. With the gradual increase in the amount of infection the animal becomes more and more anemic. It may continue in this state for two or three weeks and then gradually recover, or death may occur. Other animals, especially those experimentally infected, may die three or four days after the appearance of parasites in the blood; anemia rapidly becomes marked and the number of leucocytes and parasites is very great. Occasionally many free vermicules are observed shortly before death.

In rats with heavy infection there is marked apathy, little food is taken, and the animal sleeps constantly, with the head tucked under the thorax.

The anemia is most striking. The ears and skin, as well as the mucous membranes, become pearly white. When blood is drawn from the tail it is thin and watery, and it is very difficult to make a smooth smear. When death occurs after a long period there is great emaciation. The temperature may rise as high as 38.6° C.

Shortly before death there is often diarrhea, with an occasional admixture of blood, and the temperature is subnormal. In one animal hemoglobinuria was observed.

Lethality.—Of the 17 rats originally found infected, 4 died. However, 4 which showed severe infection were chloroformed to provide material for inoculation and fresh tissue for sectioning. Of 16 rats infected experimentally, 8 died—a lethality of 50 per cent. Young rats appear to be most susceptible and the majority of the fatal infections were in animals not fully grown. In half-grown rats the disease is almost invariably fatal in a few days after the onset of symptoms.

PATHOLOGICAL ANATOMY.

The gross lesions are most marked in animals which succumb to infection. In chronic infections the animals are much emaciated. The muscles and mucous membranes are extremely pale. The heart's blood is thin and watery. The lungs frequently show minute hemorrhages upon the surface. The spleen is greatly enlarged and dark in color; it may be five or six times the normal size, and frequently reaches from the diaphragm diagonally across to the right side of the brim of the pelvis. The small intestine occasionally contains thin fluid blood. The mesenteric glands are enlarged, sometimes hemorrhagic. The liver is distinctly enlarged, dull yellow in color, sometimes mottled, and the thin borders are rounded. Smears and frozen sections show extensive fatty changes. The kidneys show moderate parenchymatous degeneration. The urine rarely contains hemoglobin; more often bile pigment. The bone marrow is red.

THE ARTHROPODA AS AGENTS IN THE TRANSMISSION OF DISEASE.

The sexual cycle of *Hepatozoon perniciosum* takes place in a parasitic mite (an acarien), *Lelaps echidninus* Berlese. Mr. Nathan Banks, of the United States Department of Agriculture, has kindly made the determination. From a careful search of the literature it appears that this is the first recorded instance in which a member of the family *Gamasidæ* has acted as the intermediate host for a protozoan parasite.

So far as accurate knowledge extends, when a parasitic protozoon requires two hosts for the completion of its life cycle one of these hosts will be found to belong to the phylum Arthropoda. An apparent exception exists in *Hæmogregarina stepanovi* in the turtle (*Emys orbicularis*), the invertebrate host of which is a leech (*Placobdella catenigera*).[a]

Of the five subdivisions or classes into which the phylum is divided, two—the Insecta and the Arachnida—include the species which act as intermediate hosts.

Of the insects which serve as parasitic hosts and agents of transmission for pathogenic protozoa the mosquito is the best known, and the malarial parasite the only form which has been accurately worked out in all stages.

The life cycle of malaria in birds (*Proteosoma grassii*) is almost as well worked out as malaria in man. Quite recently Christophers has described the complete sexual cycle of *Piroplasma canis* in the dog tick, *R. sanguineus*.

Of insects, a number, including biting flies, lice, bedbugs, and fleas, are known to be concerned in the transmission of blood parasites. Some of these have been proved to be the agents of transmission, but whether as true intermediate hosts or merely carriers is as yet undetermined, in many instances, the sexual cycle being unknown.

In the class Arachnida the ticks have constituted, up to the present time, the only group capable of transmitting protozoan disease. The number of known tick-borne diseases is increasing and they are of great importance. In an epoch-marking work upon Texas cattle fever Smith and Kilborne,[b] in 1893, were the first to show the part played by the Arthropoda (in this instance ticks) in conveying disease. Various forms of piroplasmosis, relapsing fever, Rocky Mountain spotted fever, and *Leucocytozoon canis* have been shown to be transmissible by ticks.

Until quite recently it was believed that ticks transmit infection only through their progeny; i. e., only ticks derived from the eggs of infected mothers have power to inoculate susceptible animals. Recently it has been shown that infection with certain kinds of piroplasma is taken during the nymphal stage, but is transmitted only by the adult tick.

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[a] See p. 9, ref. [b].

[b] Smith, Th., & Kilborne: Texas fever. Bull. No. 1, Bur. Animal Indus., U. S. Dept. Agric., Wash., 1893.

PARASITIC MITES.

Since but little is generally known of the nature and structure of mites, a brief account of their habits and a detailed description of the anatomy of the mite (*Lelaps echidninus*) which is concerned in the transmission of *H. perniciosum* is given below.

The Acarina, an important group of the Arachnida, comprise the mites and ticks, the former being greatly in the majority as regards number of species. The term "mites" is often applied to the entire group of Acarina. In addition to their arachnid characteristics (the absence of antennæ and the possession of four pairs of legs) the Acarina are distinguished by the absence of a division into thorax and abdomen, and by the arrangement and structure of the mouth parts, which are adapted for puncturing and sucking. The larva resembles the parent except in the possession of three pairs of legs and the absence of sexual organs. After casting the skin two or more times (ecdysis) the adult stage, with four pairs of legs and fully formed sexual organs, is attained.

Canestrini's[a] classification, from a scheme devised by Kraemer, divides the Acarina into six subheads:

Astigmata (Sarcoptic and vermiform mites).
Hydracarina (Water mites).
Prostigmata (Harvest mites).
Cryptostigmata (Orbatid mites).
Metastigmata (Ticks).
Mesostigmata (Gamasid mites).

A number of classifications varying in slight degree have been based upon this original. The following classification of the Acarina is from Neumann:[b]

Nonvermiform Acarina	Legs inserted directly into the integument without epimeræ	Legs with five articles			*Oribatidæ*.
		Legs with six articles	With tracheæ. Cheliceræ	Provided with hooklets	*Ixodidæ*.
				Didactylous or styliform	*Gamasidæ*.
			With no tracheæ (marine forms)		*Halacaridæ*.
	Legs articulated on distinct epimeræ	Natatorial legs (aquatic)			*Hydrachnidæ*.
		Ambulatory legs	Cheliceræ styliform; palps free, antenniform		*Bdellidæ*.
			Cheliceræ styliform or in claws; palps free, ravishers		*Trombididæ*.
			Cheliceræ didactylous; palps cylindrical or conical and partially adherent to the tips		*Sarcoptidæ*.
Vermiform Acarina	Legs with five articles				*Phytoptidæ*.
	Legs with three articles				*Demodecidæ*.

Of these ten families only five are parasitic—the *Ixodidæ* (s. l.), *Gamasidæ*, *Trombididæ*, *Sarcoptidæ*, and *Demodecidæ*. Many of these are commensals, obtaining nutriment from the organic detritus.

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[a] Canestrini, S.: Prospetto dell' acarofauna Italiana., Padova, 1885–1897.

[b] Neumann, L. G.: Traite des maladies parasitaires non microbiennes des animaux domestiques. Transl. by Fleming. Ed. by J. McQueen. 2d ed. New York, 1906.

epithelial scales, hair, feathers, etc., of the host. The last two families are chiefly of interest to the veterinarian as producing various forms of mange. Only two families are blood-sucking, the *Ixodidæ* (s. l.) and the *Gamasidæ*. The rôle of the former (ticks) in the transmission of disease has already been mentioned. A few species of the *Trombididæ* are known to attack man; a larval form (*Leptus autumnalis, L. americanus*) is the well-known "red bug" or "chigger," also known as the "harvest mite" in England and the "bête-rouge" in South American countries. In Japan the Tsutsugamushi disease or Kedani disease, frequently fatal, is due to the bite of the "akamushi" or "red mite." This disease has recently attracted considerable attention; the etiology is unknown.

Among the *Gamasidæ* the "chicken mite" or "red spider louse" frequently bites those who visit houses in which fowls are kept. The *Gamasidæ* form a larger and more widely distributed family than the *Ixodidæ* (s. l.), but by reason of their small size and apparent harmlessness they have been but little considered. A number of species live on decomposing vegetable and animal matter, others act as commensals upon beetles, reptiles, birds, and small mammals. *Gamasidæ*, which are true bloodsuckers, are found upon lizards and snakes (*Ophionyssus natricis* Megnin); upon vipers and water adders they attack the cornea, rendering it opaque, the reptiles becoming blind in consequence and dying of starvation. *Dermanyssus gallinæ* is a great pest to poultry and horses and even attacks man, withdrawing blood through the punctures made by its pointed mandibles. A number of species infest sparrows, canary birds, and parrots. A great number of small mammals are affected, including rats, field mice, moles, rabbits, etc. Upon bats a very large species (*Pteroptus vespertilionis*), popularly believed to be a bedbug, is found. The number of species of *Gamasidæ* is legion, and doubtless when more attention is paid to this family many others will be discovered.

THE GAMASIDÆ.

The following brief description of the structure and habits of the *Gamasidæ* is taken in part from Banks.[a]

In general the *Gamasidæ* are broad and flat, with a chitinous integument. Eyes are absent, but numerous sensory "hairs" on various parts of the body make up for this deficiency. Many forms are rapid in movement when disturbed. The mouth parts in many species can be withdrawn into the body. The head or capitellum can also be considerably retracted. The mandibles, two in number, are usually chelate, with toothed fingers. In some mites they are styliform. In the male the mandibles usually present specific differences.

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[a] Banks, N.: A treatise on the acarina or mites. Proc. U. S. Nat. Mus., vol. 28, pp. 1–114, 1904.

Beneath the mandibles is a projection called the hypostome. Sometimes an epistome is present above the mandibles. Projecting from either side of the head or capitellum are the palpi, which are provided with sensory organs.

The inferior surface of the *Gamasidæ* is provided with a varying number of chitinous shields or plastrons, upon which are located the genital apertures. The legs are six jointed, being divided into coxa, trochanter, femur, patella, tibia, and the terminal tarsus, provided with hooks or sucking disks. The female genital opening is usually between the sternal and genital plate. In the male it is in the sternal plate.

The act of coition is very remarkable and has been described in detail by Michael.[a] Briefly, it consists in the deposition by the male of a mass of spermatozoa (often contained in a delicate cyst) into the spermatheca, a sac connected with the uterus in the female. The spermatozoa upon liberation fertilize, from time to time, the ova as they become ripened. *Gamasidæ* lay comparatively large eggs, from which, with a few notable exceptions, six-legged larvæ are hatched. In the genus *Lelaps* the eggs, when deposited, are fully one-eighth the size of the female. One egg only is deposited at a time. It contains a fully formed embryo with six legs and fully developed nervous and digestive system. Very soon after expulsion from the female the delicate membrane or shell which incloses the embryo is ruptured and a six-legged larva appears. After several moultings of the skin the adult is formed. The immature mites are termed nymphs and, according to the number of moults gone through, protonymphs, deutonymphs, tritonymphs, etc. In the nymphal stage many of the *Gamasidæ* are migratory. Often they attach themselves to beetles, mosquitoes, and other insects, it is believed for the purpose of distribution.

LIFE HISTORY OF *LELAPS ECHIDNINUS.*

The species of *Lelaps* (*L. echidninus* Berlese) found upon white rats is a true parasite and sucks the blood of the animal. Its habits differ greatly from those of ticks in that its depredations are constantly repeated and only a small amount of blood is taken at each feeding. The mite is but slightly distensible, hence its capacity is limited. The following biological observations have been made in connection with the studies on *Hepatozoon perniciosum:*

When rats are kept in a cage of some size, in which there is an abundance of straw, the mites appear to feed almost exclusively at night and during the day they leave the host and live in the damp straw where the eggs are deposited. Hence when a number of rats are in the same cage the mites may visit a different animal each night. It is an easy matter to cause the mites to get upon the rats in the daytime by stirring the straw about in the cage and making the rats move about. In small cages, such as the glass jars used in the laboratory for holding single animals, the mites behave differently, especially if sand or sawdust is placed in the bottom. The rat moving about frequently in the narrow cage keeps the mites constantly disturbed, and they remain upon their host all the time and may be found in large numbers upon the back of the animal near the root of the tail. The female mite after feeding leaves the rat to deposit her eggs, which are laid one at a time, in damp straw. Each egg contains a well developed embryo. After hatching, the six-legged larva in a few hours moults and becomes a nymph with eight legs. The nymphs, though active, are seldom found upon rats and do not appear to suck blood. The nymphs after two moultings become adult. The young adult females (at first colorless) seek the host, and draw blood from small wounds made by the maxillæ or mandibles. When fully grown they are larger and brown in color. The males do not suck blood, but feed upon animal or vegetable detritus. They are few in number upon rats. The average length of life of the female mite is about six weeks. During this period a number of feedings of rat's blood are necessary to provide material for the development of the embryos.

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[a] Michael, A. D.: British Tyroglyphidæ. Ray. Soc. I, 1901, 291 pp.; II, 1903, 183 pp.

EXTERNAL ANATOMY OF *LELAPS ECHIDNINUS.*

TECHNIQUE.—In view of the small size of the mite nearly all the details of structure of the exo-skeleton may be determined by examination with low powers of the microscope after a few minutes immersion of the live mite in alcohol.

For brief study the specimens may be mounted in water on a slide with a cover glass. For examination with higher magnifying powers mites must be macerated in caustic potash to render them more transparent. The details of the mouth parts may often be studied in live specimens to advantage, the capitellum being then more markedly protruded.

GENERAL BODY CONSTRUCTION.—The head region or *capitellum* (pl. IV) is distinctly separated from the thorax and contains the mouth parts and certain appendages. The fused thorax and abdomen are covered dorsally by the *scutum,* a single chitinous plate. On the inferior or ventral surface are three chitinous plates—*sternal, genital,* and *anal.* The sternal plate serves as a point of attachment for the legs. The latter are eight in number (four pairs) and are composed of six articles, terminating in two hooklets and a sucking disk.

The capitellum is provided with paired sharply pointed *maxillæ*, and it incases for a portion of their length the paired *mandibles*, which are provided with didactylous pinchers in the female. In the male they are modified to form "clasping" organs. The *maxillary palps* are attached, one on either side, to the lateral aspect of the capitellum. They serve as sense organs, eyes being absent, and are composed of five articles. Two *stigmata* are present, with a prolongation forward of a tubular *peritreme*. The *anus* is located in an anal plate, the anterior margin of which is convex and the posterior prolonged into a large spine. In the female the *sexual aperture* is located in the mid-line of the body in the genital plate, near the posterior border of the sternal plate. In the male the *genital pore* is in the center of the sternal plate. The body of the adult female measures from 1.1 to 0.9 mm. in length by 0.7 to 0.6 mm. in breadth. The male is two-thirds the size of the female.

INTERNAL ANATOMY OF *LELAPS ECHIDNINUS*.

TECHNIQUE.—The difficulty of dissecting so small an object as this mite, especially as it is covered with hard chitinous plates, is very considerable. With the aid of fine needles, teased preparations can be made, a small per cent of which will be fairly successful. In the main, serial paraffin sections must be relied upon. The fixatives used were 5 per cent formalin, 80 per cent alcohol containing 5 per cent of formalin, and alcohol in various strengths. In addition, a fixative composed of 3 parts chloroform, 2 parts glacial acetic acid, 1 part saturated solution of bichloride of mercury, and 6 parts absolute alcohol was employed. In some instances soaking in a solution of 2 per cent celloidin preceded the embedding in paraffin.

Some satisfactory sections parallel to the ventral surface were made without softening the chitin, since none is encountered by the knife. For other sections in which the chitinous plates are to be cut, the mites, previously exposed to the action of fixatives, must be treated with a softening solution. For this purpose Labaraques solution in varying dilutions was employed. The length of time of its action depends upon the strength of the reagent and can only be determined by trial. It was found preferable to merely soften the chitin rather than to completely remove it. In the latter event, air bubbles form in the body cavity, which greatly interfere with subsequent dehydration and embedding.

The sections, affixed to slides with Mayer's albumin fixative, were stained with Weigert's iron hematoxylin.

ANATOMY OF THE HEAD REGION.—The capitellum is prolonged anteriorly to form the mouth parts. The dorsal anterior border forms a serrated projection, the *epistome*. The mandibles, which are double jointed and provided with muscles, terminate in pinchers with serrated jaws in the female. In the capitellum they lie in chitinous sheaths and are prolonged posteriorly, to be attached by tendinous expansions to the under surface of the scutum. Muscles attached to the same point serve to retract or protrude the mandibles.

The ventral aspect of the capitellum terminates in a bifid *hypostome* or maxillæ (pl. VI). The maxillæ are situated, one on either side of the mouth opening and form part of its lateral boundary They are acutely pointed and serve as piercing organs. Between and dorsad of the maxillæ is a single elongated organ, the *lingula* or tongue (pl. VI), which presents a median groove. Its surface is covered with minute papillæ. The outline of the mouth opening is somewhat triangular, the borders being formed by fringed membranous folds.

THE PHARYNX.—The *pharynx* is situated just posterior to the mouth in which it terminates. It lies almost entirely in the capitellum forming a canal in the lower portion. It is T shaped (pl. VII, fig. 2) on cross section and its walls are connected by a series of transverse muscular bands with the chitinous covering of the capitellum. These muscles serve to pull apart the walls of the pharynx and produce suction. Interspersed with the transverse muscles are a series of circular muscular bundles (pl. VII, fig. 1) capable of constricting the pharynx.

THE ESOPHAGUS.—Posteriorly the pharynx terminates in a slender tube, the *esophagus* (pl. VII, fig. 1). This in turn communicates with the *ventriculus* or stomach. Surrounding the esophagus is a large *ganglion* or brain.

The ventriculus is a thin-walled sac consisting of a central and two lateral portions (pl. VIII, fig. 2). The former communicates anteriorly and inferiorly with the esophagus. The diverticula are prolonged anteriorly and posteriorly and communicate with the lateral aspect of the ventriculus. The wide central portion (pl. VII, fig. 1) extends from the brain to the middle third of the thorax, resting upon the inner skeletal plate. Regarding each lateral diverticulum as two projections (anterior and posterior) from the ventriculus, there are in addition two others, viz, the median anterior projection to join the esophagus and a posterior projection terminating in the *colon*, which in turn, empties into the *excretory bladder*. When the uterus contains a large embryo the ventriculus is somewhat distorted and displaced.

The ventriculus and its lateral prolongations are lined with a single irregular layer of large epithelial cells with faintly staining reticular and vacuolated protoplasm and inconspicuous nuclei. The cells rest upon a delicate basement membrane. Encircling the digestive tract are a series of minute parallel muscular fibers, each one isolated and ith a single nucleus. These fibers serve to constrict the ventriculus nd its diverticula, and in young live mites with transparent scutum ie waves of contraction, beginning anteriorly and sweeping backward, re easily discerned. Adherent to the lining layer in some places, but ften free in the lumen of the ventriculus, are large spherical cells, ometimes called "liver" cells. They possess very small faintly aining nuclei, excentrically located, and are phagocytic in nature, ften containing cell inclusions of various kinds, vacuoles, and yellow igment. According to Berlese[a] they secrete a digestive ferment nd play an important part in the digestive process. That portion f the body not taken up by the digestive and other organs is occupied y muscles which move the legs and mandibles and by fat cells.

The *colon,* a short, thin-walled tube, terminates in an *excretory ladder,* which communicates with the anus. Opening into the teral aspect of the excretory bladder are paired tubes—the *malhigian* vessels—which run forward parallel beneath the ventriculus lmost to the capitellum, to end blindly. They serve as excretory rgans, and often contain small dumb-bell shaped crystals of mineral ılt.

The *salivary* glands are paired structures in the body cavity, situated eneath the anterior extremity of the scutum (pl. vii, fig. 1). Each land is pyramidal shaped and composed of several acini, which are ormed of large cells of coarse granular structure, with small, indisnctly defined, deeply staining nuclei. The apex, which is below, rminates in a minute duct which empties into the posterior part of ie pharynx. Each acinus contains a central cavity, which varies in ze according to the activity of the gland. Small accessory glands re sometimes observed.

Sexual organs.—The *ovary* is an irregularly shaped, oval body tuated in the posterior portion of the body. It is composed of a ticular framework, in which are many young *ova* and small cells hich form the stroma. Projecting from its anterior and lateral spects are ova in various stages of development. Three or four ova advanced stages of development, one of which is fully ripened, may sually be observed.

In adult females the *uterus,* a thin-walled sac lying in front of the vary, usually contains an embryo which may show complete development. The ripe ovum is fertilized in the uterus, which communicates y a narrow vagina with the genital aperture. The latter is covered y finger-like sensory organs (pl. iv, fig. 2). Attached to the top of ie ovary are peculiar tubular structures—the *lyrate organs*—to hich are connected a pear-shaped sac (*sacculus fœmineus*) containing spermatozoa. The lyrate organs pass on either side of the uterus to terminate upon the inner aspect of the acetabula of the coxæ of the third pair of legs.

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[a] Berlese, A.: Ricerche sugli organi e sulla funzione della digestione negli acari. iv. di Pat. Veg., Terenzi; vol. 5 (5–8), 1896, pp. 129–195.

Since only the female mite is directly concerned in the transmission of *Hepatozoon perniciosum*, the anatomy of the male sexual organs will be only briefly considered. The *testis* is a single oval body situated posteriorly between the ventriculus and the excretory bladder. The paired *vasa deferentia* pass downward from each lateral aspect, then forward near the ventral surface, to terminate in the single genital opening in the center of the sternal plate. The *spermatoza* are large, round or elongated, granular structures, and are usually found in the sacculus fœmineus in the female. Here they are stored until the ova, becoming ripened one at a time, are fertilized.

Tracheal system.—The stigmata are placed on the lateral aspect of the body, between the third and the fourth pair of legs. A tubular prolongation forward, the *peritreme* or *stigmal canal* (pl. IV, fig. 2) divides into the main tracheal tubes, which ramify throughout the body, some terminating in the legs. The tracheal system is absent in the larvæ.

The following articles may be consulted for a more detailed account of the structure of the *Gamasidæ:*

Winkler, W.: Anatomie der Gamasiden. Arb. a. d. Zoolog. Inst., Wien, vol. 7, part 3, 1888, pp. 1–38.

Berlese, A.: Recherche sugli organi e sulla funzione della digestione negli acari. Riv. di Pat. Veg., Firenzi, vol. 5 (5–8), 1896, pp. 129–195.

Michael, A. D.: On the variations in the internal anatomy of the *Gamasidæ*, especially in that of the genital organs, and on their mode of coition. Trans. Linnean Soc. of Lond., 1892.

Mégnin: Memoire sur l'organization et la distribution zoologique des acariens de la famille des *Gamasides*. Journ. d. l'Anat. et de la Physiol., 1876.

Kramer, P.: Ueber Gamasiden. Arch. f. Naturg., vol. 1, 1882.

Michael, A. D.: British Tyroglyphidæ. Ray. Soc., I, 1901, 291 pp.; II, 1903, 183 pp.

SEXUAL CYCLE OF *HEPATOZOON PERNICIOSUM* IN THE MITE.

Mites removed from rats (in whose blood parasites were abundant) were found, in the majority of cases, to be infected with *H. perniciosum*. Such mites, having fed upon the rat's blood from time to time, usually showed the parasites in several stages of development. Cross sections of mites pictured in pls. IX and X show this condition. Frequently, when many fully developed parasites were present, young forms also were observed in the stomach, indicating a fresh infection with each feeding of blood. Mites which were heavily infected with fully developed parasites could often be distinguished by the naked eye from normal mites. In the former the lateral and caudal portion of the body is distended and pearly white, whereas in the latter this part is dull brown in color.

In order to determine the length of time required for the various stages of development of the parasite, as well as to study them in detail, a number of mites were artificially infected, or, more properly, the usual manner of infection was controlled. For this purpose it was necessary to have a supply of normal mites. Fortunately these could be readily obtained. The stable in which the infected rats had been kept contained a large room in which were a number of rat cages. In two corners large single isolated cages had been built. One of these contained infected rats; the other had been emptied of rats about two weeks. Upon examination both were found to contain damp straw and grain, upon which were myriads of mites. In the empty cage the mites were found to be free of infection. Six healthy rats, fresh from the dealer, were placed in this cage. In a very short time each rat was infested with a large number of mites.

To remove the mites, which run about with considerable rapidity upon the hair and skin, the rats are seized by the loose skin at the nape of the neck with the tip of a pair of long clamps. The tail of the animal is grasped with one hand and by traction the body is extended along the blades of the forceps. In this position it is easy to remove the mites with a fine-toothed comb, from which they are quickly shaken by a tap of the finger into a large glass jar. The inside of the rim of the jar should be previously smeared with a ring of vaseline, which the mites will not traverse. In this manner it is possible in a short time to collect several hundred.

The mites are kept in the jar several days to starve them and then placed upon infected rats. For this purpose four rats were selected in whose blood the parasites were very numerous. To transfer the mites to a rat it is only necessary to place the latter in the jar for a few minutes. In a short time all the mites will have crawled upon the rat and concealed themselves in the fur.

After six, twelve, eighteen, twenty-four, thirty-six, and forty-eight hours, mites were removed and examined in fresh preparations and stained smears, and a number preserved in fixing solutions. From these paraffin serial sections were made. After forty-eight hours a large number of mites were removed and placed upon healthy rats in order that the parasites then present in the mites might develop without admixtures of younger stages, which would have been the result of continued feeding upon the diseased rats. At intervals of twenty-four hours a number of these mites were removed and examined and sections made. Successive collections of mites were fed upon infected rats in order to obtain a large number of infected mites for later experiments.

In order to study the effect of the digestive juices of the mite upon encysted parasites in the rat's blood, the following experiment was performed a number of times. The various stages of the process could be conveniently followed with the microscope for an hour or more.

Blood from a rat containing numerous parasites encysted in lymphocytes was mixed with the expressed body juices of mites, placed upon a slide, and examined with the microscope. In the course of ten to thirty minutes the protoplasm of the lymphocyte was partly dissolved and the parasites were set free.

The encysted parasites, after becoming free from the leucocytes, are slender, elongated bodies, rounded at the extremities. They show no movement or change of form. After thirty minutes or longer the delicate cyst wall surrounding them is ruptured and the parasite becomes an actively motile vermicule. The remains of the cyst, as a transparent shadow, often continues to be attached to the vermicule for some time.

The same changes may be observed when a mite that has just fed upon blood is crushed and the expressed fluid examined.

When mites starved for forty-eight hours or longer are examined six hours after being placed upon heavily infected rats, numerous free vermicules are found in the stomach and its diverticula. When these mites are crushed under a cover slip in a drop of 0.3 per cent salt solution, the vermicules and a considerable number of polymorphonuclear leucocytes are observed. Other kinds of leucocytes appear to be quickly destroyed. Not infrequently the remains of the delicate cyst which inclosed the parasite is seen attached to some portion of the vermicule. In form and appearance the vermicules do not differ from those developed from the sporozoites. The movements, however, although fairly active at first, become quite sluggish, and in mites examined eighteen hours after feeding, motile forms are seldom seen.

The red blood cells disappear very quickly after the ingestion of the blood, but hematin crystals, polymorphonuclear leucocytes, and free parasites are abundant. The polymorphonuclears are especially resistant and appear to remain unchanged after a number of hours in the digestive tract of the acariens. The parasites become detached from the lymphocytes when these are digested. In mites examined twenty-four hours after feeding, the digestive fluid contains single and paired vermicules in which movement has ceased.

The form and arrangement of the paired vermicules is peculiar and characteristic. In the earliest stages they are somewhat spindle shaped and two extremities are in apposition (pl. XIII, fig. 1). This constitutes the earliest phase of conjugation or sexual union. A little later the vermicules are placed side by side and parallel, the extremities even. In appearance they are exactly similar, and hence without distinguishing sexual characters. In slightly later stages the adjoining sides are quite straight and parallel and separated by a slight interval (pl. XIII, fig. 2), although apparently attached in some manner, since their relative position is unchanged by movement of the fluid containing them. Next the two become more rounded and increase slightly in size. The nuclei are large and vesicular and the dividing line still persists (pl. XIII, fig. 3). Later one vermicule grows longer and larger and begins to partly encircle the smaller, which becomes more rounded (figs. 4–7). They may now be properly regarded as gametes. By analogy the smaller and more finely granular is the microgamete, the larger and more coarsely granular the macrogamete. Eventually the line of demarcation between them disappears. The zygote thus formed by fusion of the male and female elements, is slightly elongated, tapered at one extremity, with granular protoplasm and two large vesicular nuclei. The latter approach one another, touch, and become blended. After the union of two sexual elements in the manner described the zygote becomes more elongated (figs. 11–13) and exhibits very slow movements of flexion and extension and something resembling ameboid motion. The term ookinet is applied to such a zygote.

The ookinet continues to increase in size and becomes more and more markedly granular, especially around the nucleus, which contains a small karyosome. When first formed the ookinet measures about 10 by 25 micra. It does not appear to detach any of its substance nor does it contain pigment. From this point development is rapid. The ookinet becomes an elongated or oval body with granular protoplasm and a vesicular nucleus containing a moderate sized karyosome (fig. 13). The nuclear network is quite distinct in both fresh and stained preparations. In mites forty-eight hours after infection the ookinet has reached full size, which averages about 25 by 50 micra.

In sections of mites made at this period the ookinets are fairly numerous in the lumen of the gut and appear in many instances more or less curled up (pl. XII). A similar form is noted in fresh preparations, as well as short spindle and ovoid shapes. They often appear somewhat flattened and occasionally very long and slender. In mites, three and four days after feeding on infected rats, many of the ookinets have disappeared, having doubtless been passed out of the intestinal tract with the feces. A few, however, are adherent to the epithelium of the gut or embedded in the gut wall between the epithelial cells. The protoplasm is distinctly granular and stains much more deeply with iron hematoxylin than do the cells lining the digestive tract. The karyosome is prominent and deeply stained. Some parasites are spherical and lie directly upon the basement membrane, surrounded by epithelial cells. Others are seen outside the stomach wall in the body tissues of the mite. They are granular and deeply colored by basic stains.

The exact manner in which the basement membrane is penetrated by the parasite is unknown. Probably the contraction of the delicate muscular envelope of the alimentary sac causes the parasite to be forced through the spot, weakened by its encroachment upon the basement membrane. At any rate the ookinets are found free in the body cavity. As shown in the description of the anatomy of the mite the body cavities represent the numerous small spaces between the various organs, and between these and the chitinous body envelope. In any of these spaces, in the investing membrane of the salivary glands, or within the sheaths of the muscles inclosed in the body of the mite, the ookinets may be found.

After thus wandering about in the body tissues the ookinets become stationary and undergo a remarkable change. Coincident with an increase in size of the plasma, in which large round granules begin to appear, the karyosome becomes greatly enlarged, exceeding in size the original nucleus. The nuclear membrane is but slightly larger than this giant karyosome, which may measure 10 micra in diameter. When the parasite has reached a diameter (in the fresh specimen) of about 60 to 75 micra, it becomes oval or spherical and a delicate cyst wall makes its appearance. This stage is observed in mites four and five days after infection. It is evident that the large karyosome is the forerunner of active multiplication of the nucleus of the encysted parasite, which is now termed a sporont (Lühe) and the cyst an oocyst.

In sections of mites showing more advanced stages, the parasite often appears oval or flattened by reason of the restraining action of the various organs or tissues upon its growth. In fresh dissections of mites the sporonts are conspicuous by reason of their size, spherical form, and granular structure (pl. XIV). The cyst wall is often very much larger than the parasite it contains, the latter appearing to float freely within it. The sporonts at this stage measure from 100 to 150 micra in diameter. Upon reaching approximately this size the nucleus undergoes division into two. The division is repeated and the daughter nuclei migrate to a position near the surface of the protoplasm, which forms a perfect sphere (pl. XIV, figs. 1–2). Nuclear division continues until a large number of nuclei are formed, all located in a superficial layer of the protoplasmic mass. The oocyst reaches a maximum diameter of 200 to 250 micra, although usually somewhat smaller.

In mites infected six or seven days previously the surface of the sporont is covered with budlike projections (pl. XIV, figs. 3–4), each of which contains a nucleus. A little later the buds become more prominent and elongated and break off from the central mass. A large number (50 to 100 or more) of oval bodies are thus formed, each of which contain a nucleus. These bodies represent sporoblasts and measure about 10 by 15 micra.

When obtained in the fresh condition, by gently teasing apart mites in 0.3 per cent salt solution the sporonts are perfectly spherical or ovoidal (pl. XIV), with a delicate cyst larger than the sporont. In the large budding forms (pl. XIV) the central granular part becomes softened and gradually liquefied, the stored-up material being utilized in the growth of the sporoblasts. Very slight pressure is sufficient to rupture the sporont, causing the escape of the fluid contents and the collapse of the superficial portion with the attached sporoblasts. In sections this collapsed condition always exists, and the flattened sporonts on cross section show usually two rows of sporoblasts with the attached ends adjoining, suggesting a fern leaf in appearance (pl. XVII, fig. 5; and pl. XI).

The subsequent development of the sporoblasts is traced in various stages in pl. XVIII. The nucleus divides and subdivides, the daughter nuclei arranging themselves at the poles. A little later a cyst wall is formed around each sporoblast.

The nuclei become the nuclei of the sporozoites, the protoplasm of which is split off from the central mass as short rods (pls. XVI and XVIII), which increase in length and gradually envelop the diminishing central "rest-body," like fingers. With increasing growth of the sporozoites at the expense of the "'rest-body," the latter is eventually reduced to a small, round, granular mass. The sporozoites fill the interior of the cyst, which measures on the average 25 by 30 micra. They are arranged in two general groups, occupying the two halves of the cyst, as shown in pl. XVI, fig. 12–14, the adjoining extremities of sporozoites interlocking. This arrangement does not always persist, however, since later they may be mixed up indiscriminately and retain merely a general direction corresponding to the long axis of the cyst. When the cysts are ruptured in salt solution, the individual sporozoites appear as cresent or club-shaped bodies (pl. XVI, fig. 1–2). The nucleus, spherical in form, is located nearer the smaller extremity. The thicker and more rounded end, as will be seen later, is the anterior. The protoplasm is highly refractile. The measurements are 14 micra in average length, by 5 micra in greatest breadth, tapering to 2 micra at the narrow posterior extremity.

The rest body persists as a round granular body about 6 micra in diameter. The fully developed cysts are found in mites from ten to twelve days after feeding. The number of sporozoites is from 12 to 20 in each cyst; 16 is the average; occasionally as many as 24 are encountered. The cysts which contain the larger number are elongated and often distinctly bent in the middle. In addition to these, small spherical cysts containing about 10 sporozoites are sometimes met with. The sporozoites in such cysts do not appear to differ from those in the other cysts mentioned.

When ruptured by pressure, the cysts split in the direction of greatest length, the edges curling up. In sections of mites (pls. IX, X, XIX) showing large oocysts containing sporoblasts or sporocysts the outlines of the large cysts are somewhat irregular and not clearly outlined. Also, the varying effect of the pressure of surrounding organs and the shrinkage and distortion in fixation and embedding cause the large cysts to assume a variety of forms other than spherical.

The invasion of the salivary glands by the cysts is particularly interesting. In some specimens the gland on one or both sides has in part or entirely disappeared and been replaced by the parasites.

The sporozoites have never been found free, however, in such sections nor in fresh preparations, but always inclosed in the sporocysts. The possibility of the transmission of the parasite through the salivary glands and their appendages will be considered later.

In the study of the various stages which have just been described sections from a great number of mites and fresh preparations from hundreds of both normal and infected mites have been utilized. The observations were repeated and verified many times.

The presence of the parasites in moderate numbers does not appear to be injurious to the mites. Mites have frequently been observed, with fresh blood in the stomach and ripe sporocysts in the body tissues. However, those found dead have often been very heavily infected.

EXPERIMENTAL INFECTION IN WHITE RATS.

Although Christophers has described what he regarded as the sexual cycle of *Leucocytozoon canis* in a tick, he states in a recent volume[a] that "the sexual cycle can not yet be said to be definitely known." Certainly the experimental transmission of hæmogregarines by arthropoda or by direct inoculation has not, so far as the literature records, been successfully accomplished.

In the present work it was recognized that the conditions for experimental attempts at transmission of *H. perniciosum* were extremely favorable, the principal host being, so to speak, a domesticated animal, and therefore readily handled. The intermediate host—the mite—could easily be obtained in large numbers and its action was susceptible of complete control. In fact, all the conditions of natural infection could readily be reproduced artificially.

As already stated, a large number of mites (several hundred) were infected by being placed upon heavily infected rats for forty-eight hours or longer and then transferred to healthy rats for ten to fourteen days. When examined at the expiration of this period about 50 per cent showed heavy infection with ripe cysts. When an infected mite was crushed between slide and cover slip in a drop of salt solution hundreds of sporocysts containing sporozoites were expressed (pl. xv). Only rarely were the sporocysts still contained in the large cyst (oocyst), the latter being usually ruptured in the process of crushing or dissecting.

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[a] Stephens, J. W. W., & Christophers, S. R.: The practical study of malaria. 3d ed. London, 1908, pp. 1–414.

With a view of obtaining a clew to the manner of infection of rats in nature, a number of experiments were undertaken to determine the effect of various substances upon the sporocysts and the sporozoites. The observations were made partly upon the warm stage of the microscope, but largely without its use; the weather at the time being extremely warm, additional heat was often found unnecessary.

1. Action of fresh rat's blood.—A drop of a healthy rat's blood was mixed on a slide with ripe sporocysts, covered with a cover glass, and ringed with vaseline. No change in the sporocysts took place nor was any movement of the sporozoites in them observed as long as the sporocysts remained intact. On the contrary, when the latter were ruptured by pressure on the cover glass and the sporozoites set free in the blood, marked changes took place. After about fifteen minutes the sporozoites assumed a more elongated form, the principal change of shape taking place in the broad extremity. This end became more slender and elongated (but still slightly wider than the other) and the tip somewhat pointed. The nucleus was visible as a spherical body about one-third distant from the smaller extremity. The movement earliest observed consisted of a gradual bending until the ends were almost touching, then a sudden spring-like resumption of the original shape (pl. xvi). Other sporozoites, after becoming bent, became slowly straightened, a peristaltic wave of contraction beginning anteriorly and a gradually increasing length of sporozoite becoming straightened. A longitudinal contraction of the entire parasite, which rendered it shorter and broader, was followed by slow contraction in a transverse direction, beginning anteriorly, this portion becoming longer and thinner as the wave passed backward and causing the sporozoite to be projected slowly forward. A bending simultaneous with the longitudinal contraction often occurred, and in the following transverse contraction straightening proceeded from before backward, the anterior slender portion often making a distinct angle with the clubbed posterior part.

With high magnifying powers of the microscope and indirect light from the condenser, minute striæ, some arranged in an oblique transverse and others in an oblique longitudinal direction, can be made out without much difficulty. These striæ are caused presumably by minute fibrils—the myocyte-fibrillæ commonly observed in gregarines, which are capable of contraction.

After the slide has been allowed to remain for ten or fifteen minutes in the incubator and is then examined on the warm stage, the sporozoites are found to have become actively moving bodies called vermicules. By a sort of snakelike or wormlike motion, rapid change of place occurred, the red blood corpuscles being vigorously moved about when the vermicule came in contact with them. The anterior extremity was always the one which had been the broadest and most rounded in the sporozoite. In the moving vermicule this extremity is more slender and pointed. The entry of vermicules into leucocytes was never observed. After two or three hours they became sluggish in movement and finally stationary.

2. ACTION OF 0.5 PER CENT SOLUTION OF PANCREATIC EXTRACT IN NORMAL SALT SOLUTION.—When sporocysts are exposed to this solution the cysts are rapidly dissolved and the sporozoites set free. The latter very soon become contracted into rounded bodies and lose all motion.

3. THE DIGESTIVE JUICES OF THE MITE.—Mites containing sporocysts were compressed between a slide and cover slip. The chitinous envelope was then removed and the cover slip replaced and pressed down with sufficient force to rupture the sporocysts and set free the sporozoites. The latter, after a short time, performed slow movements of flexion and extension, and, in addition, some sporozoites showed at times a slow gliding motion forward, with intervals of rest. When in motion a minute point of protoplasm could be seen projecting from the anterior end. The protoplasm showed no perceptible waves of contraction during this movement. Some of the sporozoites at times exhibited slight contractions. No movement took place inside the unruptured cysts.

4. ACTION OF JUICE FROM THE DUODENUM OF THE RAT.—A healthy fasting rat was killed, the abdominal cavity opened, and a drop of the clear fluid contents of the lower part of the duodenum mixed on a slide with ripe sporocysts. Immediately upon examining with the microscope the sporozoites in the unruptured cysts were found to be in active motion, moving about with great vigor in the interior of the cyst and suggesting very strongly a mass of worms squirming over one another. After ten or fifteen minutes many cysts were seen to rupture and the sporozoites were set free as actively moving vermicules. They exhibited all the movements previously described as observed when sporozoites were mixed with rat's blood, except that they were intensified. This observation (repeated a number of times) upon the action of the intestinal juice upon the sporocysts and sporozoites pointed to the intestine as a possible point of entry of the infection, in rats, and led to subsequent experiments in this direction.

The first experimental attempts to produce the disease in rats were conducted with the intention of simulating the natural method of infection. Ten healthy white rats about three-fourths grown and just received from a dealer were selected. Each rat was placed in a separate glass battery jar with wire-gauze top. Careful examination failed to disclose any skin parasites except a few lice. Two blood smears were made each day on two successive days from each rat. In every rat the blood was found to be normal. Mites previously infected and showing in about 50 per cent ripe sporocysts when crushed, were placed upon eight of these rats. From 20 to 50 mites were placed upon each rat. Two rats were kept as controls, no mites being placed upon them. The rims of the jars containing these rats were smeared with vaseline and they were kept in a separate part of the laboratory away from the others. The results were as follows:

Rat No. 1.—On the nineteenth day first appearance of parasites in the blood as encysted forms in lymphocytes; leucocytes increased. Twentieth and twenty-first day: Encysted forms more numerous; animal shows marked anemia. Twenty-second day: Lymphocytes greatly increased; in addition to many encysted parasites, numerous free forms. In fresh preparations some of these are actively moving vermicules. Twenty-third day: Leucocytes 120,000, 82 per cent being large mononuclear leucocytes. In counting 200 leucocytes 381 parasites were counted. Animal died.

Post-mortem.—Muscles and skin extremely pale; blood watery; spleen greatly enlarged and dark, measuring 6 by 2 by .5 cm. Liver, yellow in color; punctate hemorrhages in lungs.

Rat No. 2.—Few encysted parasites on twenty-fifth day, gradually increasing in number until thirty-fifth day. Anemia slight. At that time 38,000 leucocytes, large mononuclears 56 per cent. In counting 200 leucocytes 32 parasites encountered. On fiftieth day infection appears to be about the same.

Rat No. 3.—Moderate number of free and encysted forms on twenty-second day. Infection increased until death on twenty-eighth day. Leucocytes, 132,000, large mononuclears 71 per cent. In counting 200 leucocytes 165 parasites observed; few free forms.

Post-mortem.—Lesions as in rat No. 1, except spleen slightly smaller and in addition there is enlargement of abdominal and mesenteric lymph glands.

Rat No. 4.—Infection first observed on twenty-eighth day; few encysted forms; gradually increasing anemia and number of encysted forms; death on thirty-eighth day. Leucocytes 110,000 on thirty-sixth day; large mononuclears 68 per cent. In counting 200 leucocytes 96 parasites observed; very few free; nucleated red cells abundant.

Post-mortem.—Lesions as in No. 1.

Rat No. 5.—Few encysted parasites on twenty-fifth day, but gradual increase up to fiftieth day. Leucocytes 28,000, 37 per cent large mononuclears. In counting 200 leucocytes 28 encysted forms observed; none free. Animal appears healthy; slight anemia.

Rat No. 6.—Few free and encysted parasites on nineteenth day. On twenty-fifth day greatly increased in number, both free and encysted; moderate anemia. On fiftieth day animal alive, but seems sick and is quite anemic. Leucocytes 98,000, 62 per cent large mononuclears; many nucleated reds. In counting 200 leucocytes 231 parasites observed; 157 free.

Rat No. 7.—Infection appeared twenty-seventh day; death thirty-first day; great anemia. On twenty-sixth day leucocytes 91,000, 59 per cent large mononuclears. In counting 200 leucocytes 101 parasites observed, mostly encysted.

Autopsy.—Spleen 6 by 1 by 0.4 cm. Other lesions as in No. 1, except punctate hemorrhages absent in lungs.

Rat No. 8.—Free vermicules only in small numbers on seventeenth day; encysted forms numerous on twenty-fifth day; death thirty-sixth day. Leucocytes 126,000, large mononuclears 78 per cent on thirty-fifth day. In counting 200 leucocytes 83 parasites, mostly encysted, were seen.

Autopsy.—Lesions as in No. 1, except slightly smaller size of spleen.

Rat No. 9—Control.—Examined every five days up to fiftieth day. No parasites found. Leucocytes 10,600, few nucleated reds.

Rat No. 10—Control.—No parasites up to fiftieth day. Leucocytes 5,800.

Control animals were repeatedly examined and found free of mites.

INFECTION IN WILD RATS.

In attempting to infect wild rats (*Mus rattus*) with *H. perniciosum* an obstacle was encountered, due to the difficulty of handling them and of keeping them healthy in captivity.

In the first experiment five large rats, averaging 250 grams in weight, were used. These rats had just been captured in a wire trap. They were placed in separate glass jars, to which infected mites had been added. Unfortunately all the rats died within a week from self-starvation and the effects of confinement.

For a second experiment six young wild rats, apparently of the same age and averaging about 100 grams in weight, were obtained. These were placed in a large wire cage, which in turn was placed in a large wooden cage containing infected mites.

Results: One rat died on the thirty-third day and another on the thirty-ninth day. Examination of the blood and organs showed severe infection with *H. perniciosum*. At the end of six weeks the remaining rats were all infected with the blood parasite, but in a mild degree. At the end of eight weeks the animals still show the parasite. The age of the rats at the beginning of the experiment is assumed to be about four months.

FEEDING EXPERIMENTS.

Ten rats were selected and placed in isolated glass jars with gauze tops. Each cage, as well as each rat, was sprinkled with "insect powder." All the rats were carefully examined and found free of vermin except a few lice. These disappeared within a few days after the "insect powder" was used.

Blood smears from each rat were examined on two successive days. The blood was normal and showed no parasites. Before the feedings with infected material all food was withdrawn for forty-eight hours. Mites containing ripe sporozoites were crushed singly or in small numbers at a time upon double-width glass slides. Immediately after crushing, examination with the low power of the microscope was frequently resorted to, in order to be certain of the abundance of ripe sporocysts. Pieces of moistened white bread were used to absorb the expressed juices of the crushed mites. After a thin piece of bread about 2 cm. square had received from 15 to 20 or more crushed mites and their juices it was gently compressed into a firm round mass and immediately fed to a rat. In this manner eight of the ten rats were fed. The other two were kept as controls. The eight rats were fed as described only once; afterwards ordinary food (bread and oats) was given.

Twenty-four hours after the infected bread was given, and every day thereafter, stained blood smears, and on occasion, fresh preparations, were examined. In the first twenty-four hour preparations, five rats showed a moderate number of actively moving free vermicules in the blood; they were also observed in stained smears and differed in no way from those previously described. The leucocytes were but little if at all increased in number; no encysted or other variety of parasites were observed. When next examined (third day) no parasites were observed in any of the smears examined. The later findings are given below:

Rat No. 1.—A small number of encysted parasites observed on the eighth day; on twelfth day moderate anemia; parasites greatly increased, also leucocytes; death on fourteenth day.

Post-mortem.—Enlarged spleen, fatty liver.

Rat No. 2.—Moderate numbers of encysted and a few free parasites observed on twelfth day; gradual increase in number up to fortieth day; animal shows moderate anemia and seems sick; encysted parasites numerous.

Rat No. 3.—Moderate number of encysted parasites on eighth day; tenth day marked anemia, many free and encysted parasites. Found dead on morning of twelfth day.

Post-mortem.—Hemorrhagic spots on surface of lungs; blood in small intestine; other lesions as in No. 1.

Rat No. 4.—Few encysted parasites on ninth day; moderate number on twelfth day. On fortieth day an occasional parasite observed; animal appears healthy.

Rat No. 5.—Small number of free and occasional encysted parasites on eighth day; tenth day encysted forms numerous; moderate anemia; death on twentieth day.

Post-mortem.—Spleen greatly enlarged; liver mottled yellow; many parasites in blood.

Rat No. 6.—Encysted parasites in small numbers on twelfth day; moderately numerous on twentieth day; animal sick. On fortieth day a few encysted forms still present; animal appears healthy.

Rat No. 7.—Few free and many encysted parasites on ninth day; more abundant on twelfth day; animal anemic, appears sick; leucocystes greatly increased. Increasing anemia and death on twentieth day.

Post-mortem.—Spleen four times normal size; liver enlarged and fatty; mesenteric lymph glands enlarged and hemorrhagic.

Rat No. 8.—Small number of encysted parasites on eleventh day; on fifteenth day considerably increased; anemia apparent. On fortieth day animal still alive, but quite sick and anemic.

Rats Nos. 9 and 10—Control rats.—Were examined in the same manner as the infected ones. No parasites found in the blood at any time. Animals healthy on the fortieth day.

On none of the rats were mites or other ecto-parasites found. The jars in which the rats were confined were placed at some distance in the laboratory from those containing infected mites and were further protected by a ring of vaseline.

In a later experiment, carried out in the same manner, five rats were used. Four were fed with ripe cysts on wet bread. The earliest appearance of encysted parasites in the blood was on the eighth day in two rats; in the other two on the tenth day. Vermicules alone were observed in three on the second day. One rat died on fifteenth; the other on nineteenth day.

In a still later experiment four young rats (2 months old) were fed as in the previous experiments. Encysted parasites were found in the blood on the tenth, twelfth, thirteenth, and fourteenth day, respectively. All four died within a week after the appearance of infection.

Remarks.—Parasites spoken of as "encysted" refer to those forms inclosed in cysts and usually contained in mononuclear lymphocytes.

In the first experiment the five rats in which free vermicules were observed twenty-four hours after feeding infected mites were those in which encysted parasites were first observed. It is probable that a more prolonged search for parasites in the blood of the remaining three rats would have resulted in the finding of a few at that time.

An attempt was made to infect four rats by feeding them livers from infected rats in which cysts (containing merozoites) were numerous. The result was negative. No free vermicules were detected in the blood during the first twenty-four hours, nor did any encysted parasites appear later.

Defibrinated heart's blood from an infected animal was injected into the peritoneum of two healthy rats, 1 c. c. to each rat. Blood smears previously made from the infected rats showed only encysted parasites. Result negative.

In a later experiment defibrinated blood in which there were a few free vermicules was employed. The results were also negative.

Small pieces of liver containing ripe cysts were gently rubbed up in a mortar with salt solution. The liquid, strained through sterilized gauze, was injected into the peritoneum of two rats. Result negative.

Several mites containing ripe sporocysts were gently crushed and rubbed in a mortar containing 0.3 per cent salt solution. The liquid, when strained and examined with the microscope, was found to contain many free sporozoites. A portion of the fluid was injected into the peritoneal cavity of two rats. Result negative.

Action of citrate solutions upon encysted parasites.—Defibrinated blood from an infected rat was added in varying amounts to small test tubes containing 1 c. c. of 1 per cent solution of sodium citrate in normal salt solution. Two tubes were set aside at room temperature (about 30° C.); two tubes were placed in the incubator at 37° C. for twenty-four hours. The same method of procedure was also followed, using normal salt solution instead of soidum citrate solution. Results: In salt solution kept for three days at 30° C. a few sluggishly motile vermicules were found. In other solutions no change took place in the encysted parasites.

A series of sections were made from the middle and lower part of the small intestine of a fasting rat, fed twelve hours before, on a large number of crushed mites heavily infected with ripe sporocysts, inclosed in moist bread. Numerous vermicules were seen between the epithelial cells and in the capillaries and lymph spaces in the villi.

CLASSIFICATION.

Some zoologists have favored a classification of the gregarine-like blood parasites as a subdivision of the gregarines, while others have placed them in a subdivision of the *Hæmosporidia*, making blood parasitism the principal feature upon which to base the classification.

Just at present the question of their systematic position is in a somewhat confused state.

In the classification of Laveran[a] the *Hæmosporidia* are divided into three genera:

1. *Hæmamœba* (including the malarial type of organism).
2. *Piroplasma.*
3. *Hæmogregarina.*

Neveu-Lemaire[b] believes that these primary subdivisions should be into families instead of genera. He divides the *Hæmosporidia* into four families instead of three, viz:

Hæmogregarinidæ	*Lankesterella.* *Karyolysus.* *Hæmogregarina.*
Hæmamœbidæ	*Plasmodium.* *Laverania.* *Hæmamœba.*
Halteridiidæ	*Halteridium.* *Polychromophilus.*
Achromaticidæ	*Achromaticus.* *Dactylosoma.*

It has been suggested that *Piroplasma*, an important group which has been left out, be included as an additional family.

Lühe agrees with Neveu-Lemaire that the genus *Hæmogregarina* (Laveran) should be given the strength of a family.

The classification of Minchín[c] attempts to accomplish the same end in a somewhat different manner. It is a modification of one proposed by Labbé.

Hæmosporidia	*Hæmosporea*	*Lankesterella.* *Karyolysus.* *Hæmogregarina.*
	Acystosporea	*Plasmodium.* *Laverania.* *Hæmoproteus.* *Halteridium.* *Piroplasma.*

The description of the suborder *Hæmosporea* is as follows:

> Trophozoite, typically a vermiform hæmogregarine, endoglobular in early stages, free when full grown; apparently no alteration of hosts; schizogony and sporogony in the same host, which is always a cold-blooded vertebrate, fish, amphibian, or reptile.

The hæmogregarines of mammals could not be included in this suborder without considerable modification. The classification devised by Neveu-Lemaire and advocated by Lühe divides the *Hæmogregarinidæ* into three genera, as already mentioned.

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[a] Laveran, A.: Les hématozoaires endoglobulaires. C. R. soc. biol., Par., vol. 50, 1899, p. 124–133.

——: Essai de classification des hématozoaires endoglobulaires. C. R. soc. biol., Par., vol. 53, 1901, p. 798.

[b] Neveu-Lemaire: Les hématozoaires du paludisme. Thèse, Paris, 1901.

[c] Minchín: The sporozoa. In "A treatise on zoology," ed. by E. Ray Lankester, part 1, London, 1903.

In view of the restricted knowledge of many of the parasites of this general group, and especially in view of the fact that the complete life cycle for so few forms is definitely established, considerable difficulty naturally arises in attempting to definitely classify *Hepatozoon perniciosum*, the organism under discussion. As Lühe, who has recently published a revisional discussion of this group, has classified an apparently rather closely allied species, namely, *Hæmogregarina balfouri*, from *Jaculus jaculus* in the family *Hæmogregarinidæ*, it seems best to place this new species for rats at least provisionally in the same family.

In attempting to classify the organism in one of the new genera we are faced by two dangers: In the majority of the species of this group the complete life cycle has not yet been established. If, now, the organism under discussion is classified in one of these new genera the danger is present of giving a total misconception to the life cycle of the other forms. If, on the other hand, a new genus is proposed for this species the danger is present that when the life cycle of the other species of this group shall become known this new genus will be shown to be unnecessary. A choice must therefore be made between running the risk of confusing the biology of species with which the present parasite may have nothing whatever to do and of unnecessarily creating a new systematic name. When these considerations are weighed it would appear that it is much more difficult to correct a biological confusion than it is to suppress a synonym. On this account it is believed that the lesser of the two evils lies in proposing for the species under discussion a new generic name. Dr. Charles Waddell Stiles, chief of the division of zoology, Hygienic Laboratory, Public Health and Marine-Hospital Service has suggested the provisional formation of a new genus. The writer accepts this view, and accordingly proposes *Hepatozoon*, kindly suggested by Doctor Stiles as the new mono-typic genus, with *Hepatozoon perniciosum* as type species.

As only one species is at present established for this genus it is naturally difficult to separate the generic from the specific characters. As diagnosis for *Hepatozoon perniciosum* n. g., n. sp., the following may be given:

In the Rat.

When an infected mite is swallowed by a rat the sporocysts are acted upon by the digestive juices and the sporozoites are liberated. The sporozoites become actively motile striated vermicules measuring 16 by 4 micra. They penetrate the intestinal villi of the rat, enter the blood stream, and are carried to the liver. They penetrate the liver cells and undergo schizogony. The nuclei of the oval schizonts are arranged at the poles and an enveloping cyst is developed. Each nucleus becomes the basis of a merozoite, of which an average of 16 are formed, arranged at the poles. The ripe cyst measures 25 by 30 micra. Later the cyst wall becomes softened and is ruptured. The merozoites are set free as actively moving vermicules, indistinguishable from those developed from the sporozoites. Some of the merozoites enter fresh liver cells and repeat the schizogony. Other merozoites enter the blood streams as vermicules, are taken in by the large mononuclear lymphocytes, and develop a definite enveloping cyst wall. A few vermicules may escape the action of the leucocytes and appear in the blood stream.

In the Mite.

When the blood of an infected rat is swallowed by a mite the encysted trophozoites are set free in the stomach by solution of the cyst as free vermicules. Two similar vermicules become associated and conjugate. One, the macrogamete, grows larger and partly surrounds the other, the microgamete. The protoplasm becomes fused and later the nuclei conjugate and fuse to form a zygote. The zygote becomes a sluggishly motile ookinet, which penetrates the stomach wall of the mite and enters the body tissues and becomes encysted (oocyst). Here a remarkable enlargement of the karyosome takes place. The parasite increases enormously in size. The nucleus of the spherical sporont thus formed undergoes division into many daughter nuclei, which migrate to the surface of the sporont. The surface of the latter becomes mammillated. The projections, each of which contains a nucleus, increase in size and length; later they are broken off and each becomes a sporoblast. The nucleus of the sporoblast undergoes division, the resulting nuclei being arranged at the poles. The sporoblast increases in size and a cyst wall develops. Around each nucleus a sporozoite is formed. In the ripe sporocyst, which measures 25 by 30 micra, the sporozoites, 16 in number (average), are arranged at the poles. The large cyst (oocyst) contains from 50 to 100 of such sporocysts. When the mite is swallowed by a rat the cycle is repeated.

It is recognized that the hæmogregarines *Leucocytozoon canis* and *Leucocytozoon funambuli*, which have already been described, in India are more or less similar to *Hepatozoon perniciosum;* but as the genus *Leucocytozoon* has already been taken by a blood parasite, *L. ziemanni*, of an entirely different character, this generic name can not be properly retained.

The relation or possible identity of *Leucocytozoon ratti*, described by Adie, with *H. perniciosum* can only be cleared up when the life cycle of the former is known. Only the trophozoite has been observed, which, according to the description, is smaller than that of *H. perniciosum*, and is without a cyto-cyst. It may be mentioned that there exists a marked similarity in form of the trophozoites of different species of hæmogregarines in both warm- and cold-blooded animals.

Specimens of the type species of the new genus have been deposited in the museum of the division of zoology, hygienic laboratory, United States Public Health and Marine-Hospital Service.

CONCLUSIONS.

Hepatozoon perniciosum is the only hæmogregarine occurring in mammals which has been observed outside of India and northern Africa, both tropical regions.

No other hæmogregarine has been described which exhibits distinct pathogenic properties or causes the death of the animal host.

Other hæmogregarines have been found only in wild animals, in which they appear to exert no harmful effect. It is well known that domesticated animals may succumb to infection by protozoa or bacteria, practically harmless to closely related wild animals. Such may be the case as regards infection of white rats by *H. perniciosum*.

The results thus far obtained in the experimental infection of wild rats (*Mus rattus*) with this parasite are not conclusive as regards pathogenicity. That the wild rat is susceptible to infection is clearly shown. As is the case with white rats, it is probable that the most pathogenic effect is exerted upon young animals. Whether or no this parasite can be turned to account in the destruction of wild rats is a matter for further investigation. The role assumed by wild rats as agents in the transmission of plague makes it a matter of great importance to exterminate them. To do this by spreading disease among them is generally regarded as the simplest and most desirable method.

The Gamasid mite (*Lelaps echidninus*), which is the intermediate host, has frequently been found both upon white and wild rats and in widely separated localities. A study of the habits of rats taken in conjunction with the experimental infections already described, proves beyond question the mode of entry of *Hepatozoon perniciosum* into the body of the rat. That this is the sole manner in which infection takes place is not proved, but is extremely probable.

The puncture produced by the pointed mouth parts of the mite before sucking the blood of the host apparently causes some irritation. Rats infested with mites may frequently be seen to bite at the skin on various parts of the body and frequently they seem to catch and devour the offending mite. The behavior of dogs when disturbed by fleas is more familiar.

If the mite in question has previously sucked blood from a diseased animal and sufficient time has elapsed for the development of sporocysts, the latter, when dissolved in the intestinal juice of the rat, set free the sporozoites. These as vermicules penetrate the intestinal wall, enter the veins and lymphatics, and reach the liver. Here they penetrate the liver cells and undergo multiplication. Some escape into the general circulation. The merozoites when fully developed are set free in the liver capillaries as vermicules. The majority are speedily taken up by the phagocytic large lymphocytes and become encysted; a few may remain as free vermicules.

The habit of mites to leave the rat during the day and return at night affords ample opportunity for a change of hosts if several animals are in the same cage.

As shown by experiment, in rats fed upon infected mites mixed with bread, free vermicules appear in the blood twenty-four hours later and encysted forms after eight or ten days, whereas rats upon which live infected mites are placed show infection only after seventeen to twenty-eight days. The explanation of this difference doubtless depends upon how soon after being placed on the rat an infected mite is devoured.

As already mentioned in the description of the parasite in the body of the mite, there is no indication, either in preparations of fresh mites or in prepared sections, that the sporozoites become free from the sporocysts in the body of the mite or that they are transmitted by the mouth parts. The sporocysts show considerable tenacity and resistance to rupture. Although sometimes encountered in the salivary glands, the sporocysts are common in any part of the body of the mite.

Infection through the alimentary canal from an intermediary host has not previously been described for a protozoan blood parasite.

The fact that all of those animals, viz, frogs, snakes, lizards, turtles, etc., in which hæmogregarines are so common, are insectivorous suggests a possible mode of infection the same as in white rats. *Leucocytozoon canis*, the sexual cycle of which Christophers has described in the dog tick, may possibly be transmitted in the same manner as *H. perniciosum.*

The limited experiments seem to indicate that infection with the latter by inoculation from one animal to another is impossible.

LIST OF HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH AND MARINE-HOSPITAL SERVICE.

The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, located in Washington, was authorized by act of Congress, March 3, 1901.

The following *bulletins* [Bulls. Nos. 1–7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.] have been issued:

*No. 1.—Preliminary note on the viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau.

*No. 3.—Sulphur dioxid as a germicidal agent. By H. D. Geddings.

No. 4.—Viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 5.—An investigation of a pathogenic microbe (*B. typhi murium* Danyz) applied to the destruction of rats. By M. J. Rosenau.

*No. 6.—Disinfection against mosquitoes with formaldehyd and sulphur dioxid. By M. J. Rosenau.

No. 7.—Laboratory technique: Ring test for indol, by S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis; Microphotography with simple apparatus, by H. B. Parker.

By act of Congress approved July 1, 1902, the name of the "United States Marine Hospital Service" was changed to the "Public Health and Marine-Hospital Service of the United States," and three new divisions were added to the Hygienic Laboratory.

Since the change of name of the Service the bulletins of the Hygienic Laboratory have been continued in the same numerical order, as follows:

*No. 8.—Laboratory course in pathology and bacteriology. By M. J. Rosenau. (Revised edition March, 1904.)

*No. 9.—Presence of tetanus in commercial gelatin. By John F. Anderson.

No. 10.—Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States. By Ch. Wardell Stiles.

*No. 11.—An experimental investigation of *Trypanosoma lewisi*. By Edward Francis.

*No. 12.—The bacteriological impurities of vaccine virus; an experimental study. By M. J. Rosenau.

*No. 13.—A statistical study of the intestinal parasites of 500 white male patients at the United States Government Hospital for the Insane; by Philip E. Garrison, Brayton H. Ransom, and Earle C. Stevenson. A parasitic roundworm (*Agamomermis culicis* n. g., n. sp.) in American mosquitoes (*Culex sollicitans*); by Ch. Wardell Stiles. The type species of the cestode genus *Hymenolepis;* by Ch. Wardell Stiles.

No. 14.—Spotted fever (tick fever) of the Rocky Mountains; a new disease. By John F. Anderson.

No. 15.—Inefficiency of ferrous sulphate as an antiseptic and germicide. By Allan J. McLaughlin.

*No. 16.—The antiseptic and germicidal properties of glycerin. By M. J. Rosenau.

*No. 17.—Illustrated key to the trematode parasites of man. By Ch. Wardell Stiles.

*No. 18.—An account of the tapeworms of the genus *Hymenolepis* parasitic in man, including reports of several new cases of the dwarf tapeworm (*H. nana*) in the United States. By Brayton H. Ransom.

*No. 19.—A method for inoculating animals with precise amounts. By M. J. Rosenau.

*No. 20.—A zoological investigation into the cause, transmission, and source of Rocky Mountain "spotted fever." By Ch. Wardell Stiles.

No. 21.—The immunity unit for standardizing diphtheria antitoxin (based on Ehrlich's normal serum). Official standard prepared under the act approved July 1, 1902. By M. J. Rosenau.

*No. 22.—Chloride of zinc as a deodorant, antiseptic, and germicide. By T. B. McClintic.

*No. 23.—Changes in the Pharmacopœia of the United States of America. Eighth Decennial Revision. By Reid Hunt and Murray Galt Motter.

No. 24.—The International Code of Zoological Nomenclature as applied to medicine. By Ch. Wardell Stiles.

No. 25.—Illustrated key to the cestode parasites of man. By Ch. Wardell Stiles.

No. 26.—On the stability of the oxidases and their conduct toward various reagents. The conduct of phenolphthalein in the animal organism. A test for saccharin, and a simple method of distinguishing between cumarin and vanillin. The toxicity of ozone and other oxidizing agents to lipase. The influence of chemical constitution on the lipolytic hydrolysis of etheral salts. By J. H. Kastle.

No. 27.—The limitations of formaldehyde gas as a disinfectant with special reference to car santitation. By Thomas B. McClintic.

No. 28.—A statistical study of the prevalence of intestinal worms in man. By Ch. Wardell Stiles and Philip E. Garrison.

*No. 29.—A study of the cause of sudden death following the injection of horse serum. By M. J. Rosenau and John F. Anderson.

No. 30.—I. Maternal transmission of immunity to diphtheria toxine. II. Maternal transmission of immunity to diphtheria toxine and hypersusceptibility to horse serum in the same animal. By John F. Anderson.

No. 31.—Variations in the peroxidase activity of the blood in health and disease. By Joseph H. Kastle and Harold L. Amoss.

No. 32.—A stomach lesion in guinea pigs caused by diphtheria toxine and its bearing upon experimental gastric ulcer. By M. J. Rosenau and John F. Anderson.

No. 33.—Studies in experimental alcoholism. By Reid Hunt.

No. 34.—I. *Agamofilaria georgiana* n. sp., an apparently new roundworm parasite from the ankle of a negress. II. The zoological characters of the roundworm genus *Filaria* Mueller, 1787. III. Three new American cases of infection of man with horsehair worms (species *Paragordius varius*), with summary of all cases reported to date. By Ch. Wardell Stiles.

*No. 35.—Report on the origin and prevalence of typhoid fever in the District of Columbia. By M. J. Rosenau, L. L. Lumsden, and Joseph H. Kastle. (Including articles contributed by Ch. Wardell Stiles, Joseph Goldberger, and A. M. Stimson.)

No. 36.—Further studies upon hypersusceptibility and immunity. By M. J. Rosenau and John F. Anderson.

No. 37.—Index-catalogue of medical and veterinary zoology. Subjects: Trematoda and trematode diseases. By Ch. Wardell Stiles and Albert Hassall.

No. 38.—The influence of antitoxin upon post-diphtheritic paralysis. By M. J. Rosenau and John F. Anderson.

No. 39.—The antiseptic and germicidal properties of solutions of formaldehyde and their action upon toxines. By John F. Anderson.

No. 40.—Miscellaneous zoological papers. By Ch. Wardell Stiles and Joseph Goldberger.

No. 41.—Milk and its relation to the public health. By various authors.

No. 42.—The thermal death points of pathogenic micro-organisms in milk. By M. J. Rosenau.

No. 43.—The standardization of tetanus antitoxin. An American unit established under authority of the act of July 1, 1902. By M. J. Rosenau and John F. Anderson.

No. 44.—Report No. 2 on the origin and prevalence of typhoid fever in the District of Columbia, 1907. By M. J. Rosenau, L. L. Lumsden, and Joseph H. Kastle.

No. 45.—Further studies upon anaphylaxis. By M. J. Rosenau and John F. Anderson.

No. 46.—*Hepatozoon perniciosum* (n. g., n. sp.); a haemogregarine pathogenic for white rats; with a description of the sexual cycle in the intermediate host, a mite (*Lelaps echidninus*).—By W. W. Miller.

In citing these bulletins, beginning with No. 8, bibliographers and authors are requested to adopt the following abbreviations: Bull. No. ——. Hyg. Lab., U. S. Pub. Health & Mar.-Hosp. Serv., Wash., pp. ——.

MAILING LIST.

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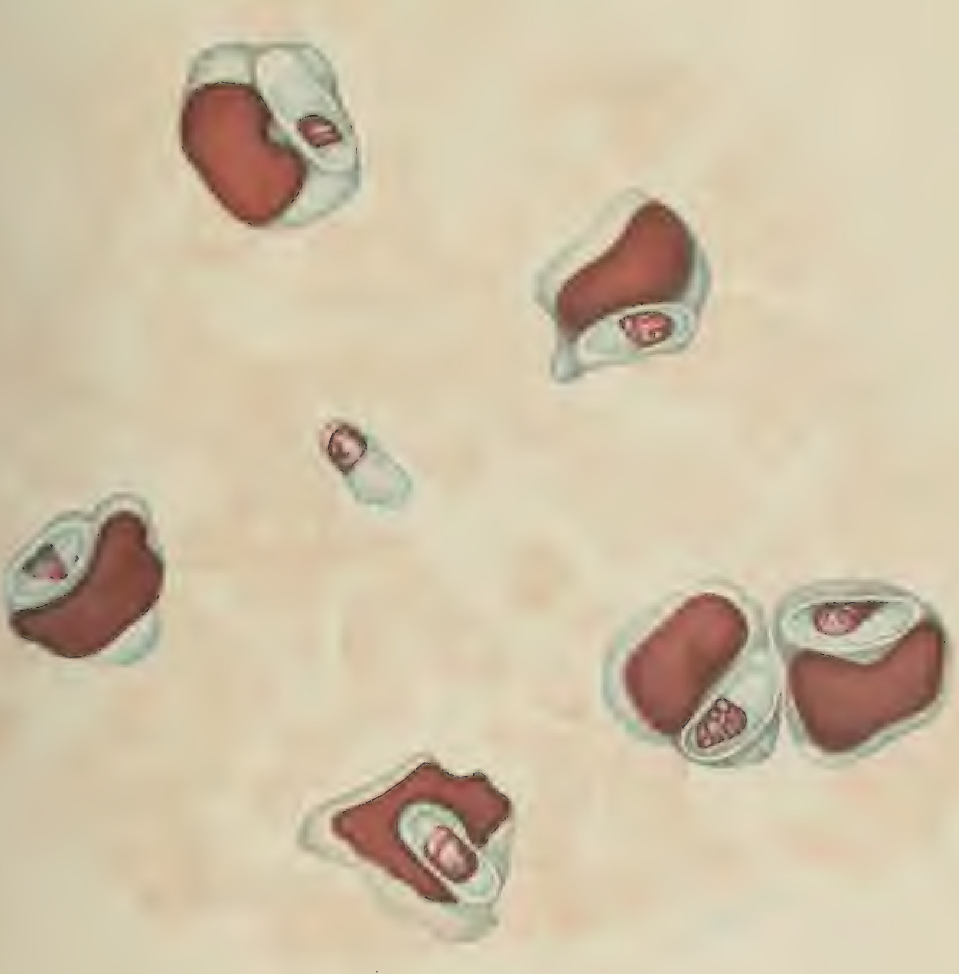

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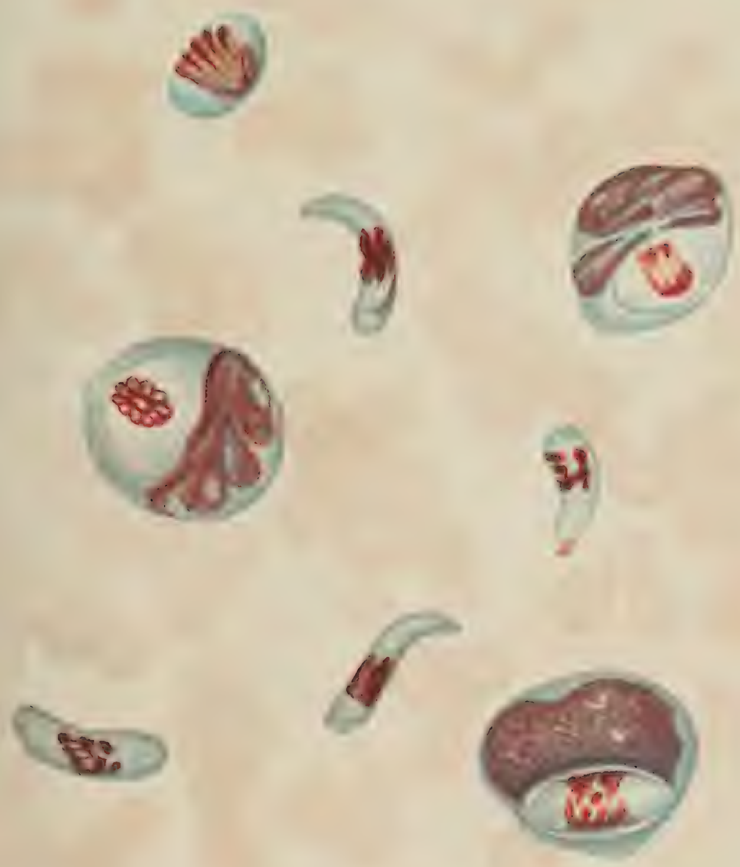
Blood smear showing *Hepatozoon perniciosum* encysted in large lymphocytes; also, one free encysted form. Drawn with the aid of the camera lucida from a single field of the microscope. Giemsa stain.

PLATE II



A Hoen & Co. Baltimore.

Blood smear showing unusual forms of *Hepatozoon perniciosum*. *a.* free vermicules; *b.* motionless, free parasites; *c.* large oval parasite without cyst; *d.* large parasite in transitional leucocyte, unencysted; *e.* ordinary size parasite in transitional leucocyte, without cyst; *f.* large parasite encysted in large mononuclear lymphocyte.

Drawn with camera lucida. Giemsa stain.

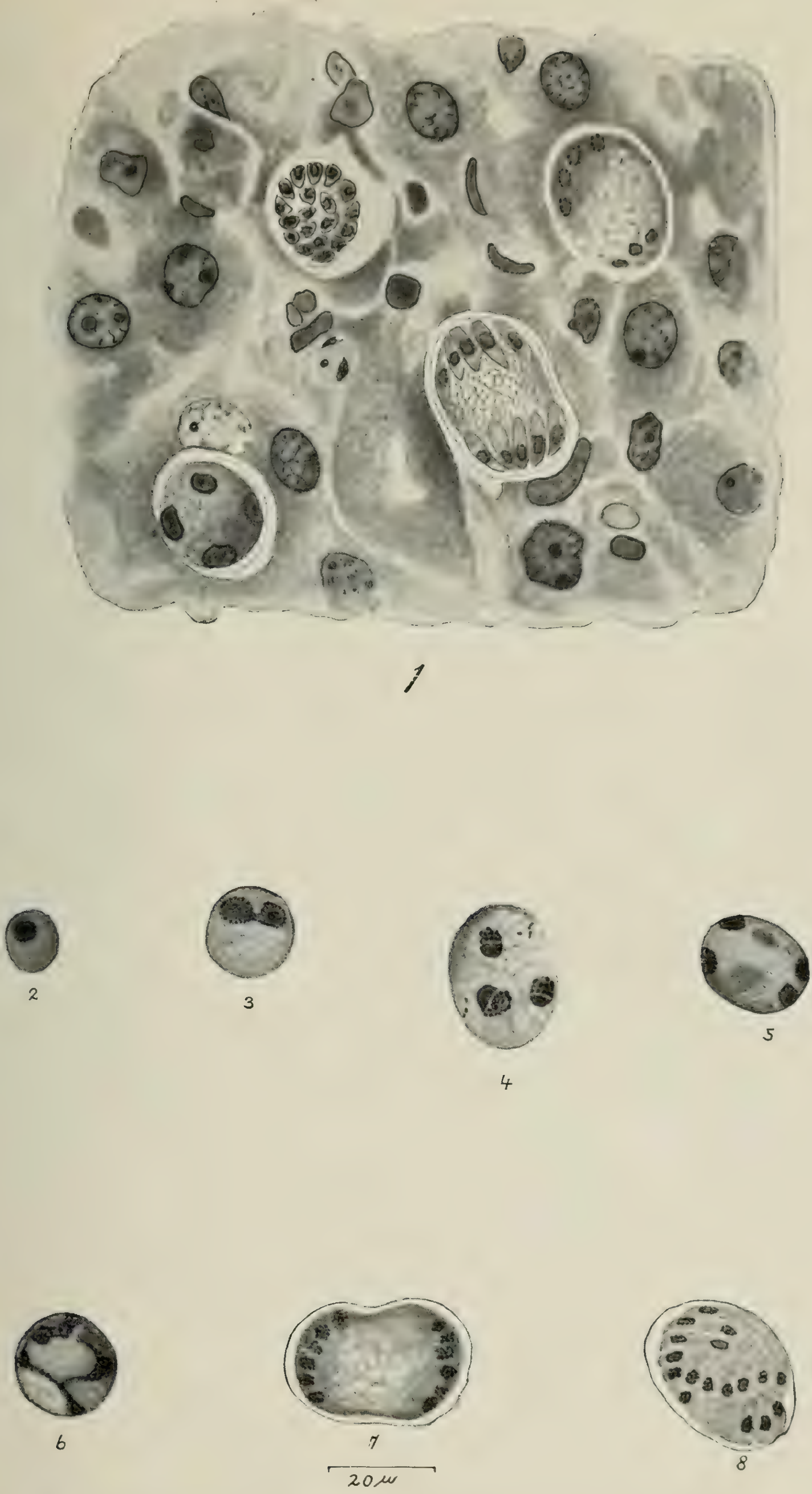
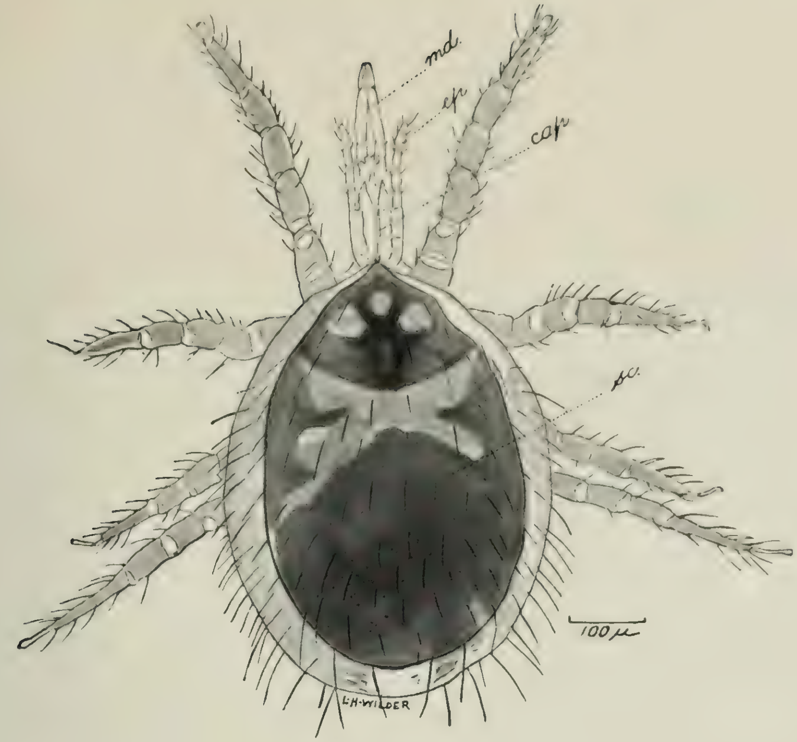


Fig. 1.—Section of liver of infected rat, showing schizonts in various stages and the formation of merozoites. Figs. 2–8.—Detail drawings of schizonts in different stages (from a section of the liver). Drawn with camera lucida. Stain, iron hematoxylin.



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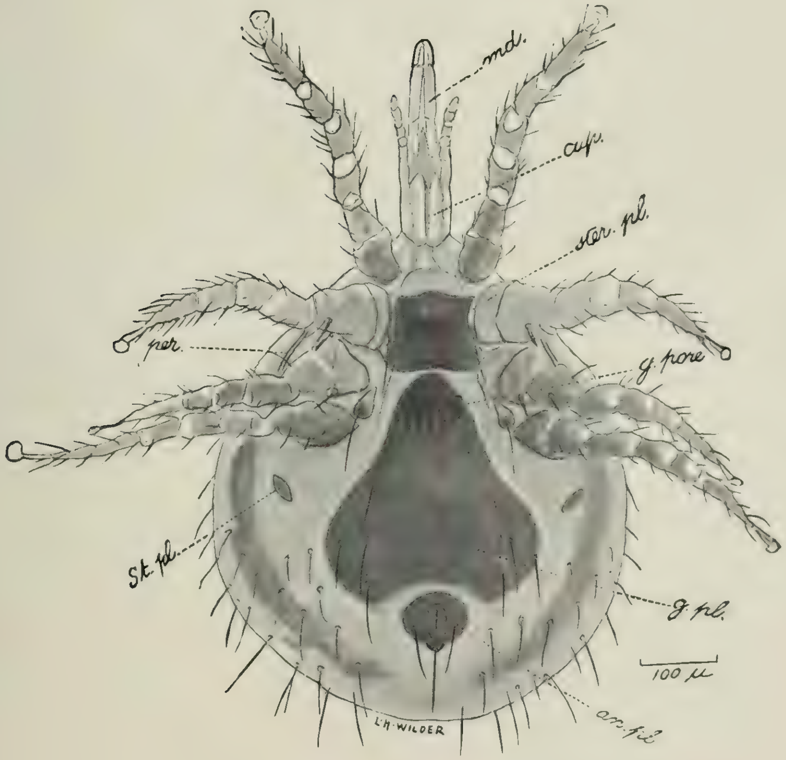
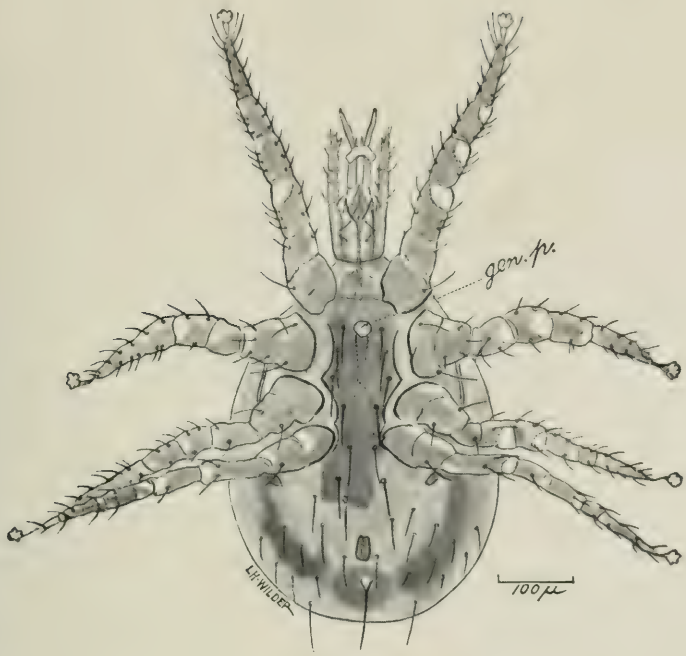


2

Fig. 1.—Dorsal view of a female mite. Fig. 2.—Ventral view of a female mite. *md.*, mandibles; *ep.*, epistome; *cap.*, capitellum; *sc.*, scutum; *per.*, peritreme; *st. pl.*, stigmal plate; *g. pl.*, genital plate; *an. pl.*, anal plate; *g.*, genital pore; *ster. pl.*, sternal plate. Drawn with camera lucida.



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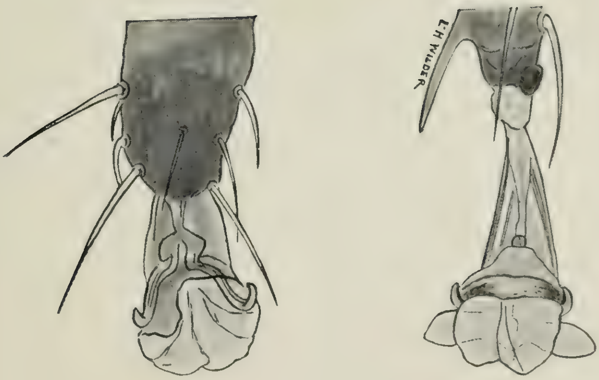
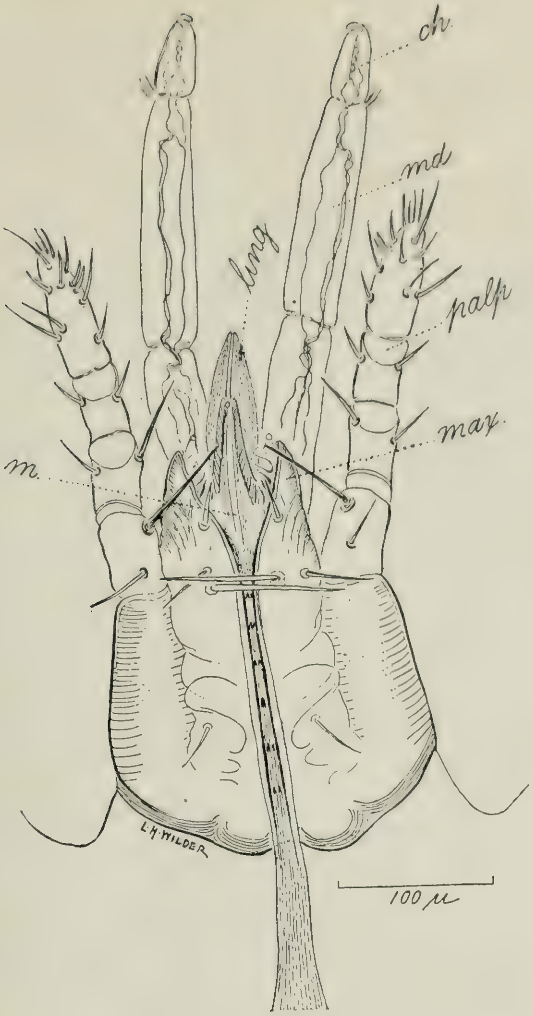


2

Fig. 1.—Ventral view of male mite. *gen. p.*, genital pore. Drawn with camera lucida. Fig. 2.—Sucking disks and hooklets upon terminal phalanx of first pair of legs. Greatly enlarged.



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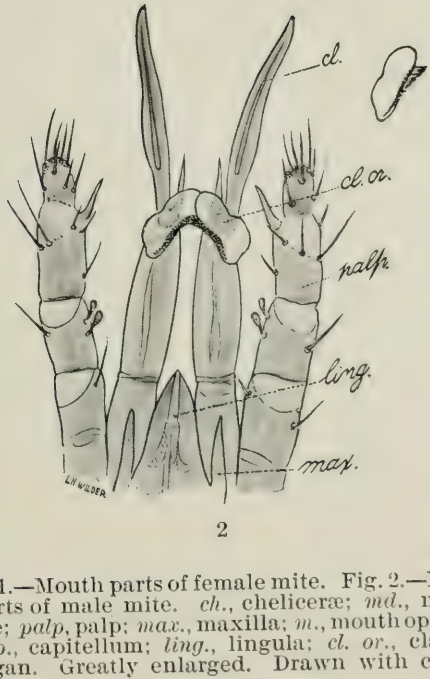
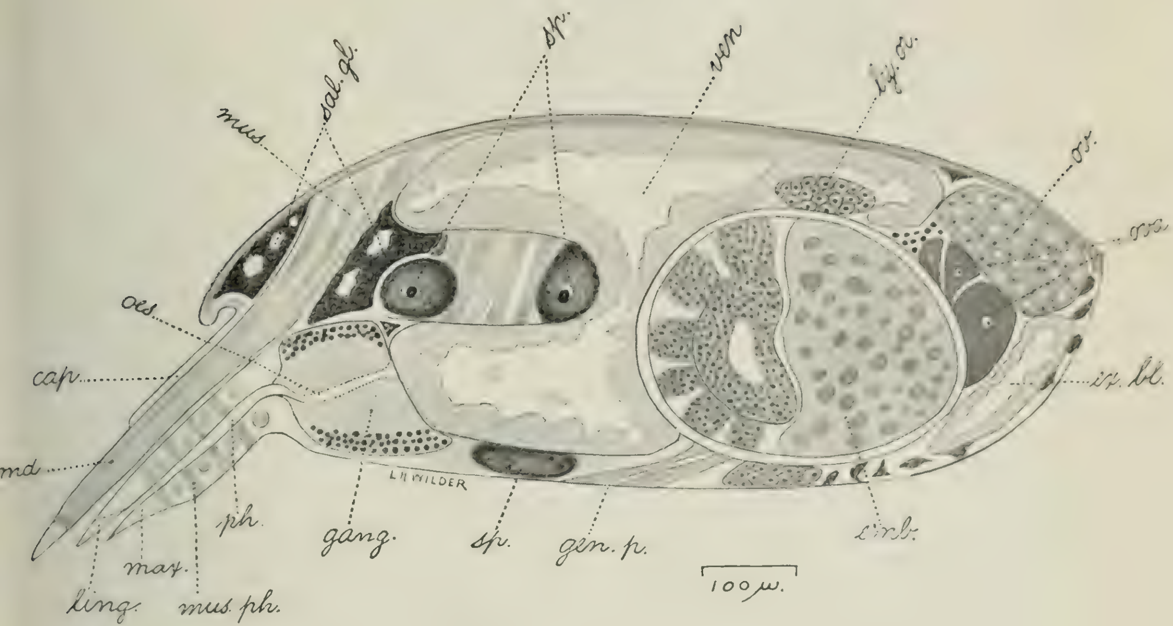


2

Fig. 1.—Mouth parts of female mite. Fig. 2.—Mouth parts of male mite. *ch.*, cheliceræ; *md.*, mandible; *palp*, palp; *max.*, maxilla; *m.*, mouth opening; *cap.*, capitellum; *ling.*, lingula; *cl. or.*, clasping organ. Greatly enlarged. Drawn with camera lucida.



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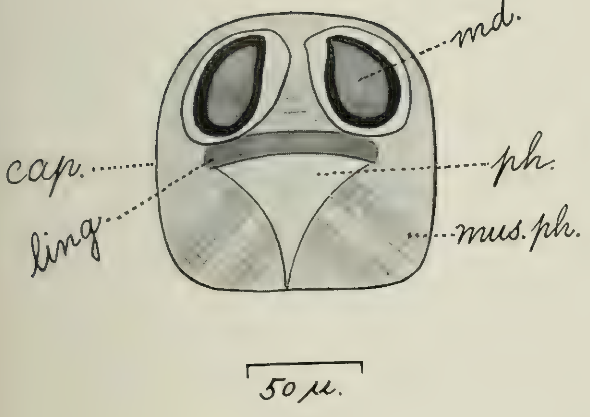

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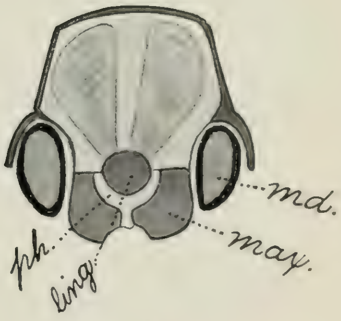
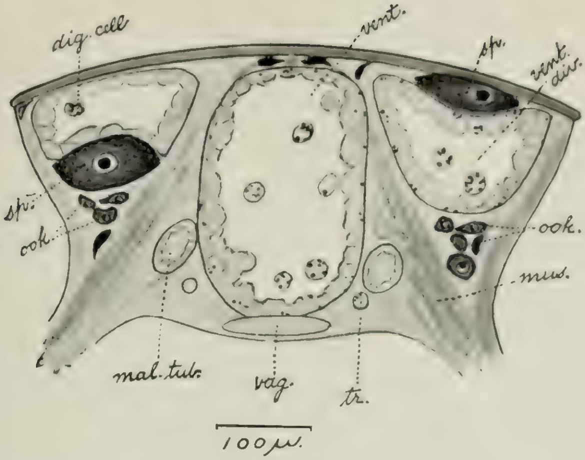


3

Fig. 1.—Dorsi-ventral section in mid line of female mite. Semidiagrammatic. Fig. 2.—Transverse section of posterior part of capitellum. Fig. 3.—Transverse section of anterior part of capitellum (enlarged). *ven.*, ventriculus; *cap.*, capitellum; *ov.*, ovary; *ova*, ova; *emb.*, embryo; *gang.*, ganglion or brain; *gen. p.*, genital pore; *œs.*, œsophagus; *sal. gl.*, salivary gland; *ly. or.*, lyrate organ; *mus.*, muscle fibers (attached to mandible, and under surface of scutum); *md.*, mandible; *max.*, maxilla; *ex. bl.*, excretory bladder; *ling.*, lingula; *ph.*, pharynx; *mus. ph.*, pharyngeal muscles; *sp.*, sporont (parasites).



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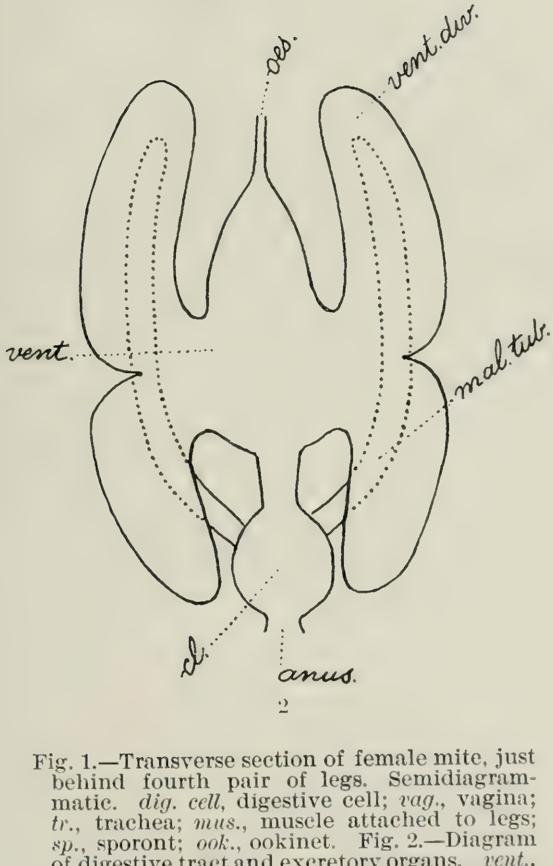
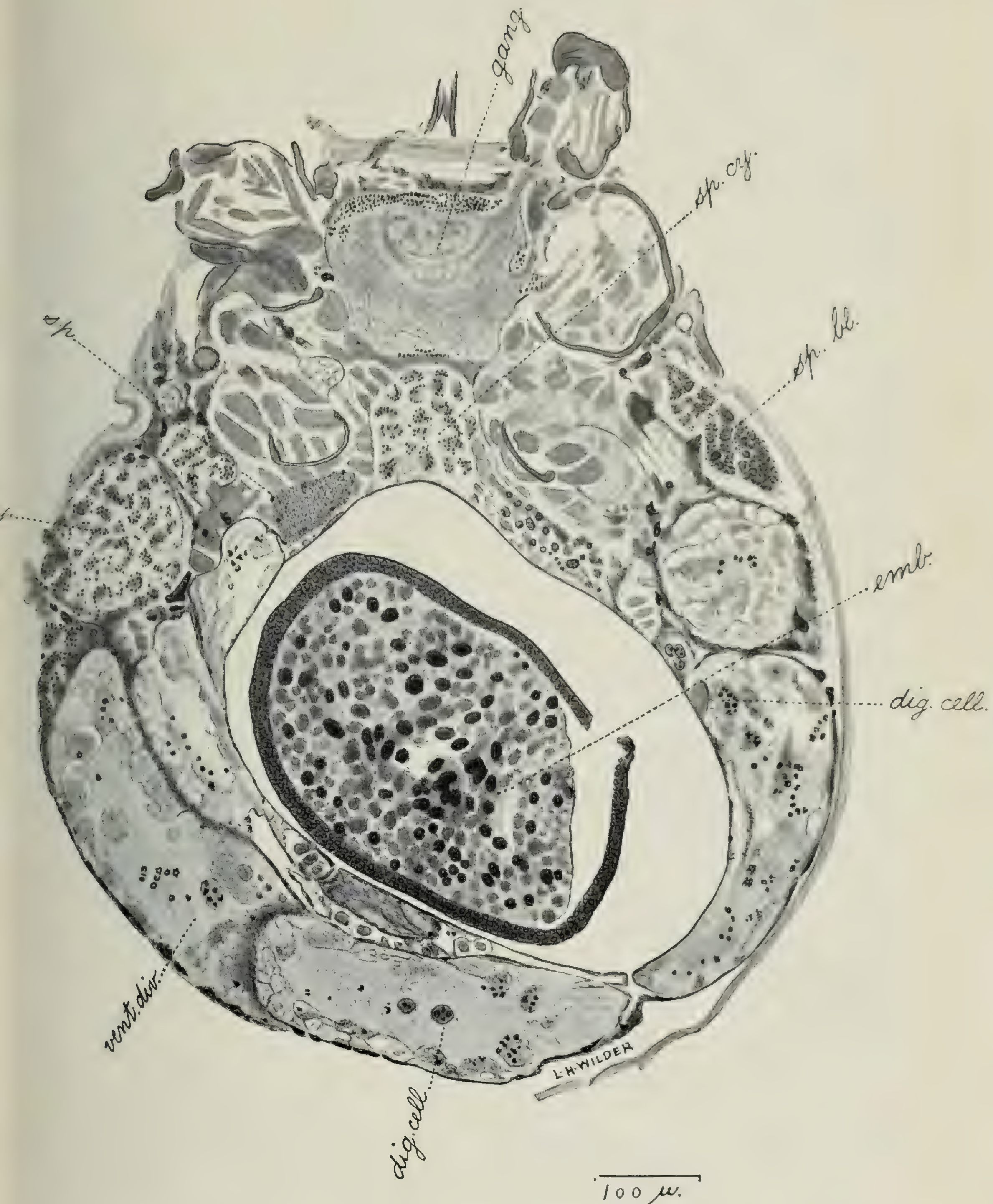
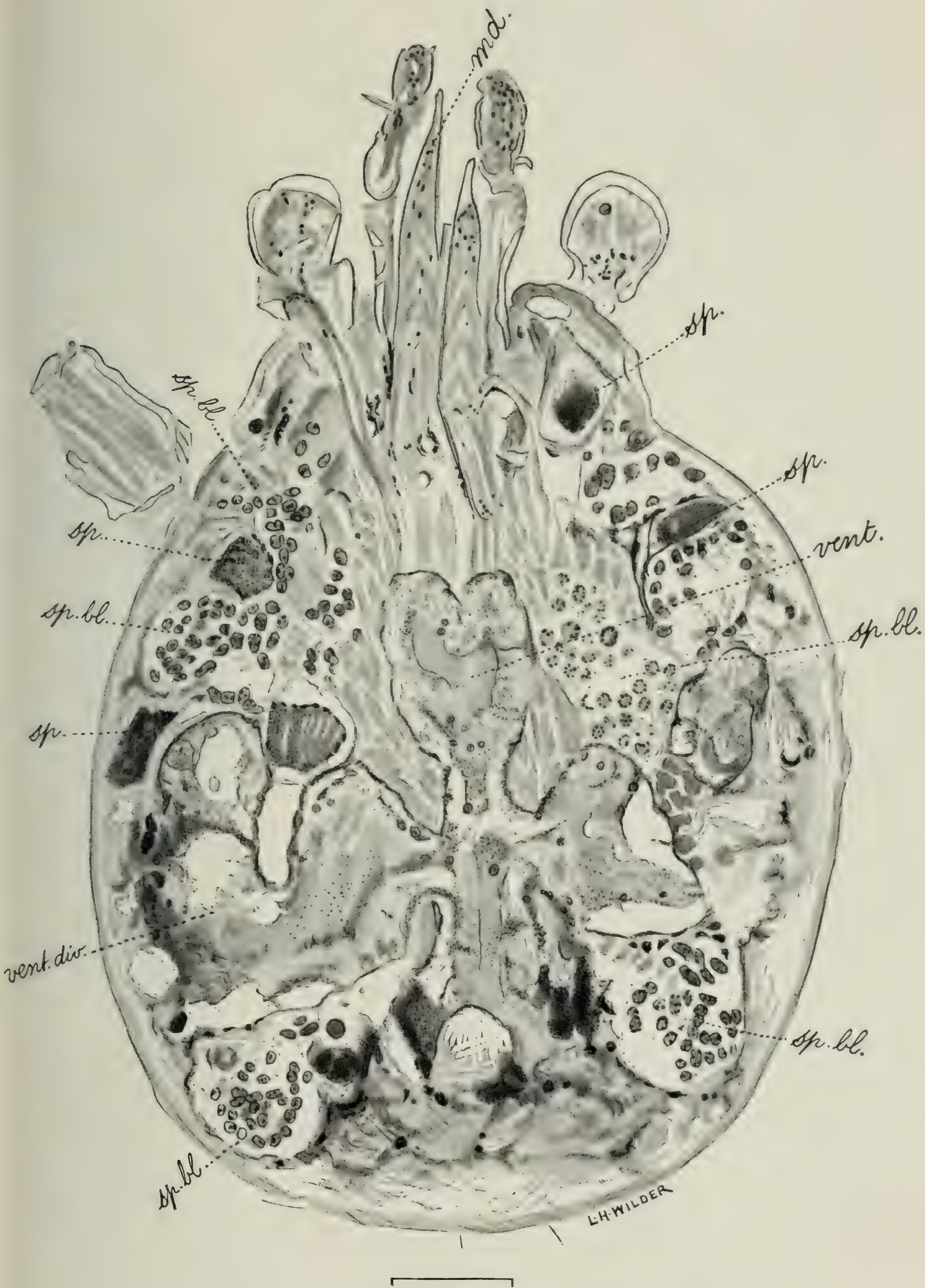


2

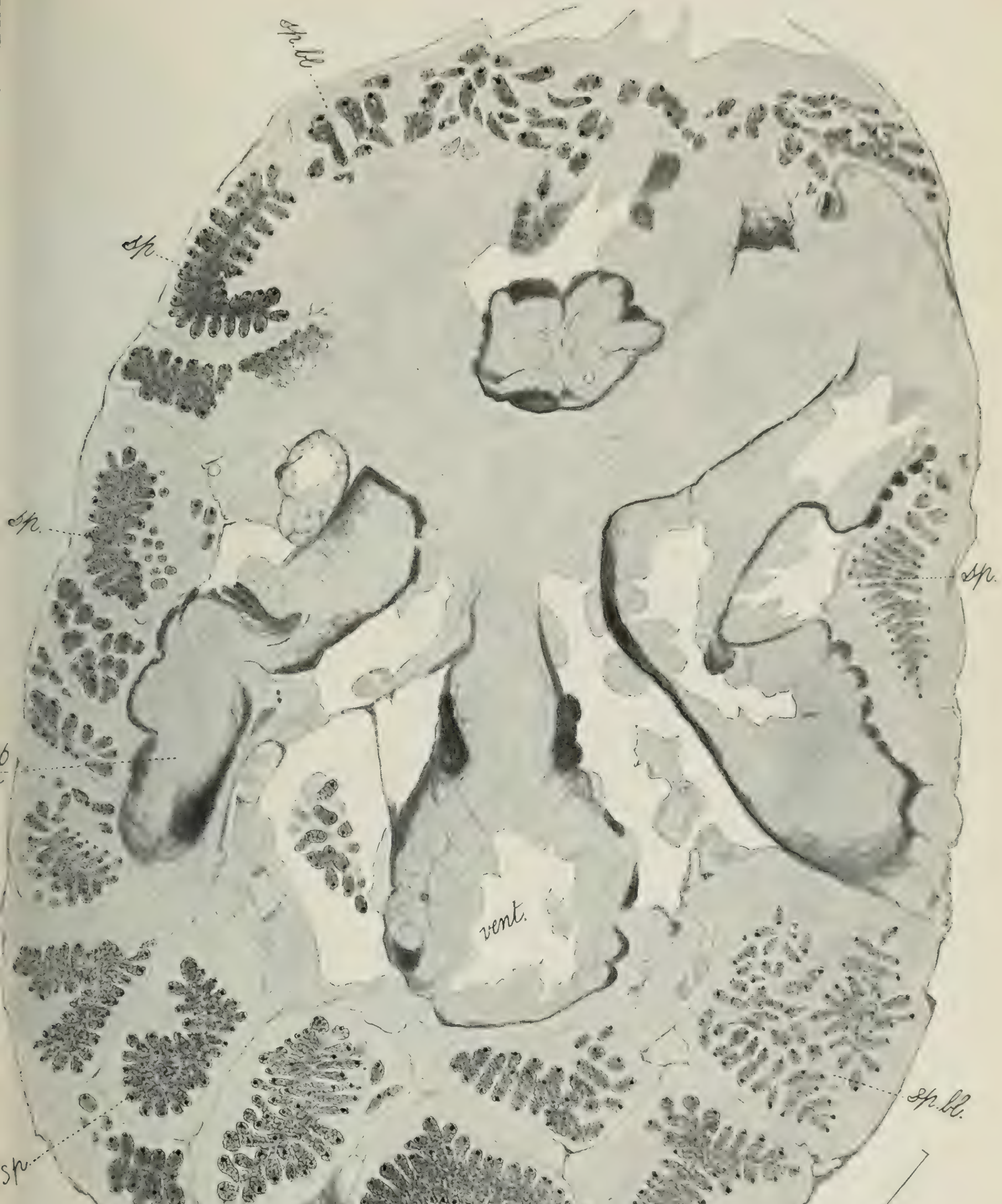
Fig. 1.—Transverse section of female mite, just behind fourth pair of legs. Semidiagrammatic. *dig. cell*, digestive cell; *vag.*, vagina; *tr.*, trachea; *mus.*, muscle attached to legs; *sp.*, sporont; *ook.*, ookinet. Fig. 2.—Diagram of digestive tract and excretory organs. *vent.*, ventriculus; *vent. div.*, ventricular diverticulum; *mal. tub.*, malphigian tubule; *cl.*, excretory bladder; *œs.*, œsophagus.



Horizontal section of female mite, removed from an infected rat. *sp. cy.*, large cyst containing many sporocysts with sporozoites—the cyst walls do not show distinctly—the nuclei of the sporozoites are strongly stained; *sp.*, sporont showing peripheral nuclei; *sp. bl.*, large cyst containing sporoblasts; *gang.*, ganglion or brain; *vent. div.*, ventricular diverticulum; *dig. cell*, digestive or liver cell; *emb.*, embryo. Drawn with camera lucida. Stained with iron hematoxylin.



Horizontal section of young female mite from an infected rat, showing parasites. *sp. bl.*, sporoblasts (not yet divided into sporozoites) in large cysts, with indistinct outline; *sp.*, sporont. Lettering as in Pls. V to IX. Drawn with camera lucida. Stained with iron hematoxylin.



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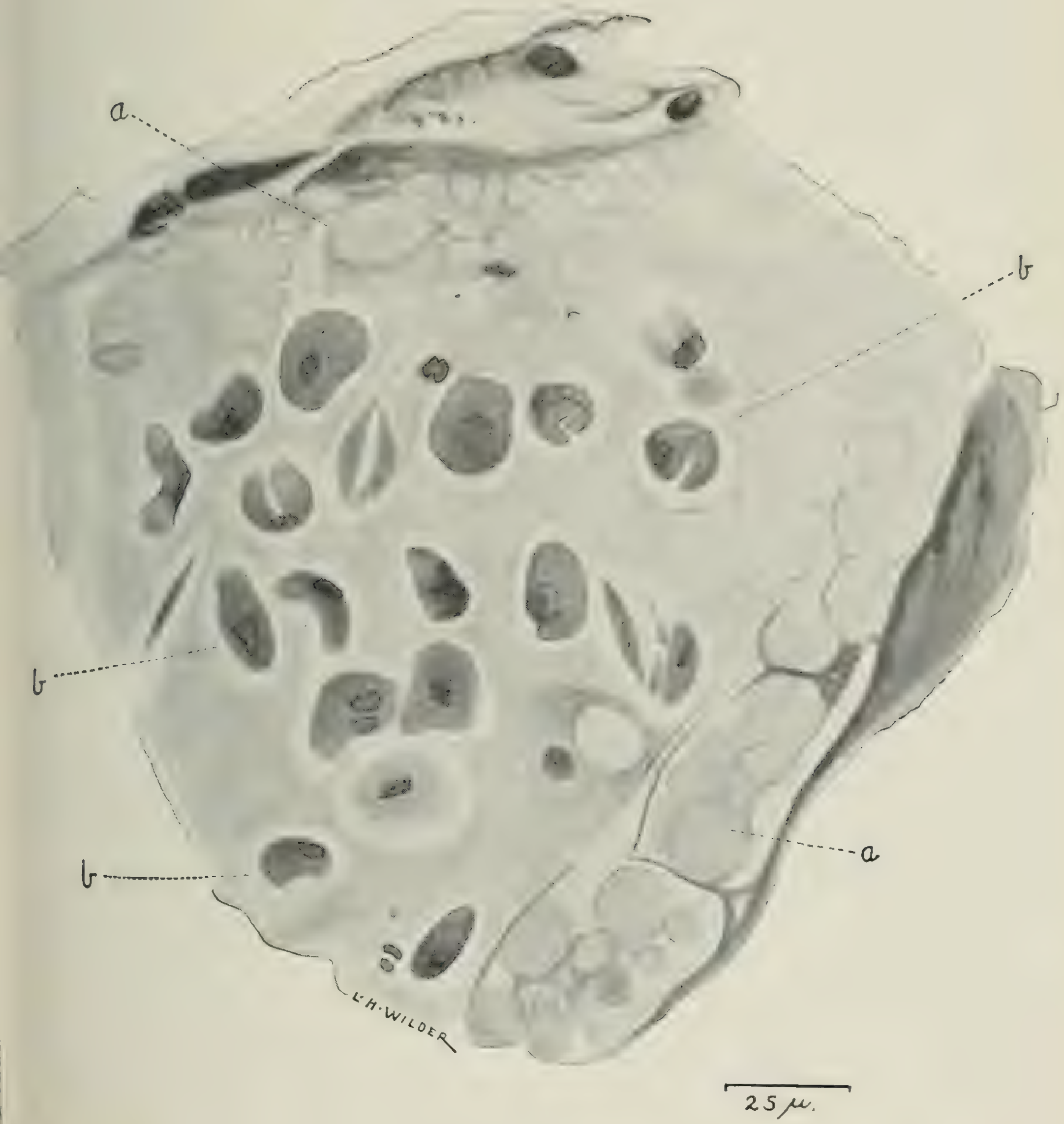
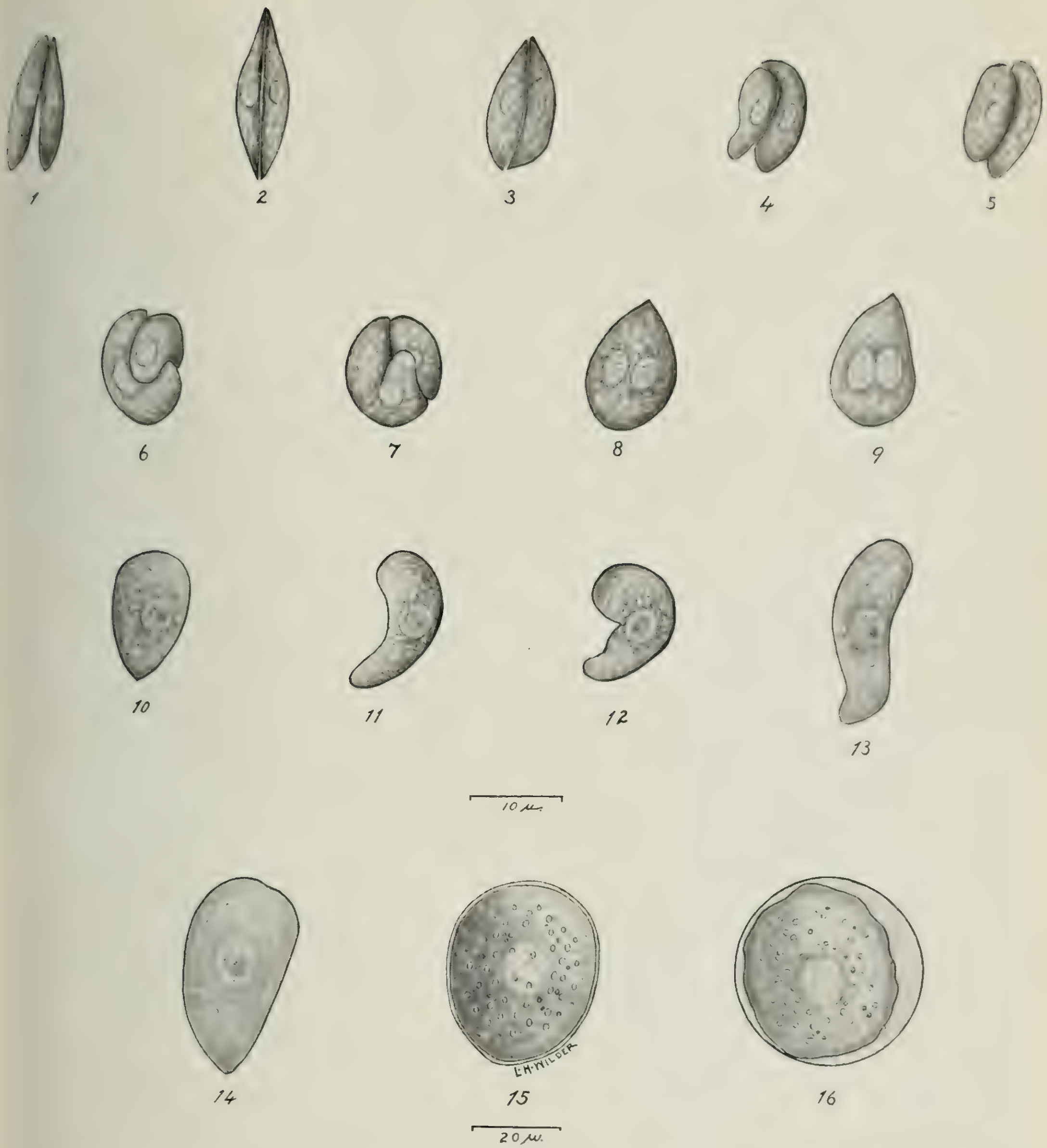
Horizontal section of young female mite, from a lot placed upon a heavily infected rat for 24 hours, then removed and placed upon a normal rat; about seventh day; section shows enormous number of segmenting sporonts. The large cyst surrounding each sporont is irregular in outline. Some of the sporonts are shown completely divided up into sporoblasts. Drawn with camera lucida. Stained with iron hematoxylin.



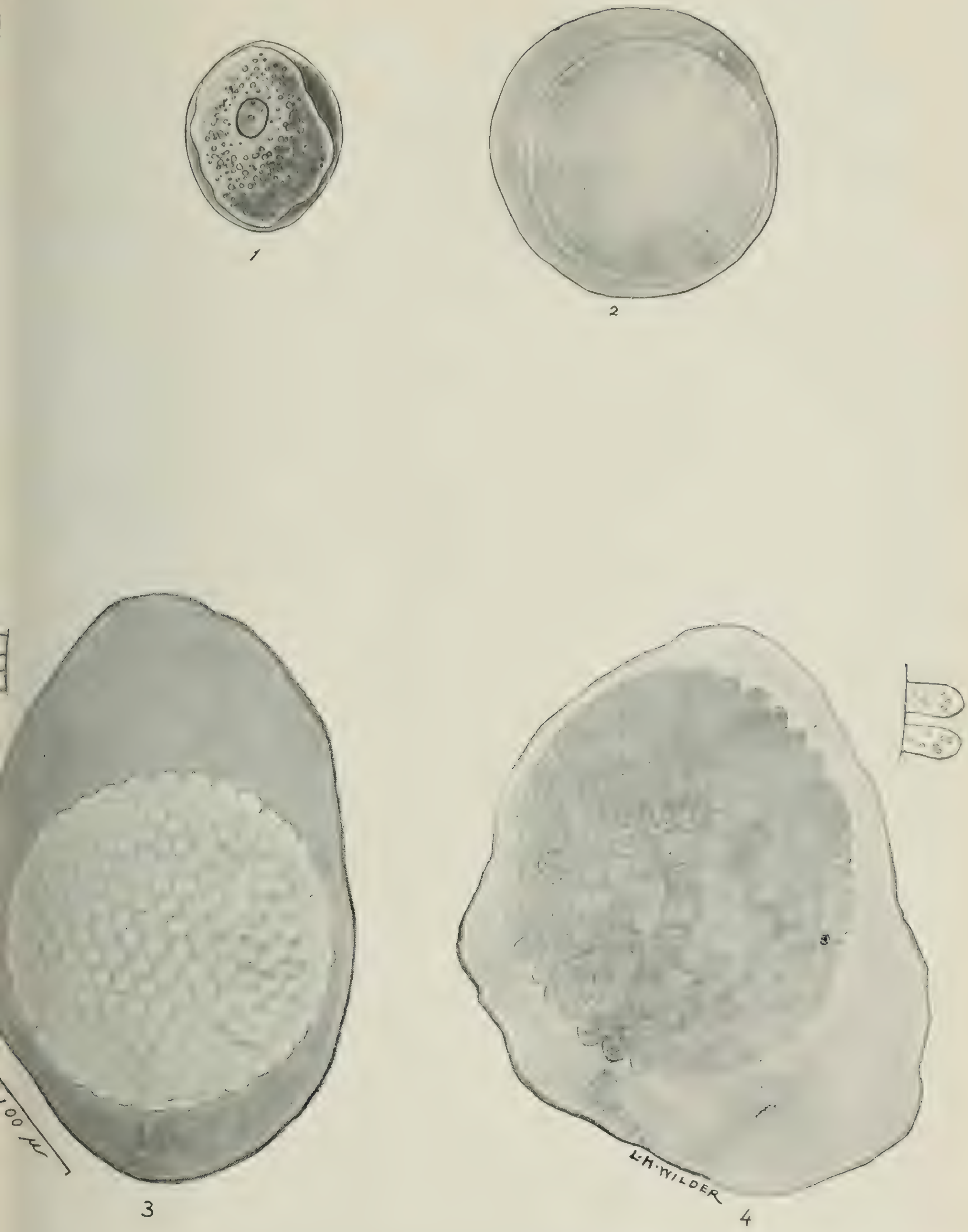
From a horizontal section of a mite 48 hours after feeding upon an infected rat. Figure represents the anterior portion of the left lateral diverticulum of the ventriculus in section. Stained with iron hematoxylin. *a*, Epithelial and muscular layer of ventriculus; *b*, zygotes and ookinets of various forms. Drawn with camera lucida.



SEXUAL PHASE OF HEPATOZOON PERNICIOSUM IN THE STOMACH AND BODY TISSUES OF A FEMALE MITE.

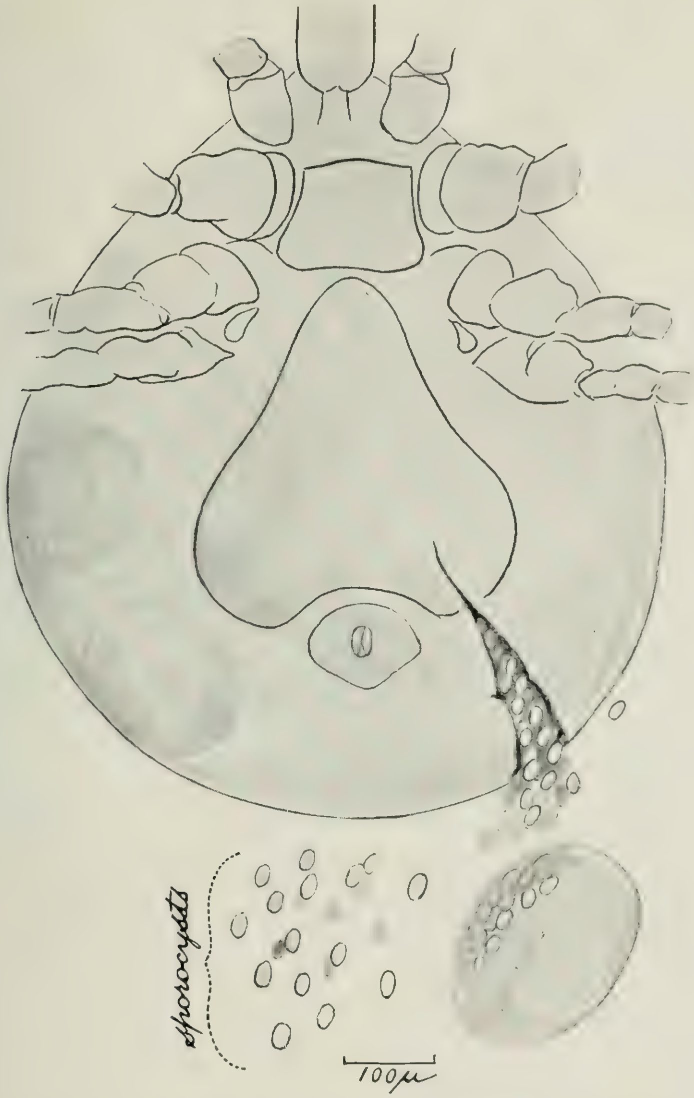
Fig. 1.—Association of vermicules (gametocytes). Figs. 2-5.—Conjugation of gametes. Figs. 6-9.—Copulation of gametes to form a zygote. Figs. 10-14.—Formation and development of ookinet. Figs. 15-16.—Formation of an enveloping cyst and beginning of sporont stage. Figs. 1-12.—In the ventriculus. Figs. 13-16.—In the body tissues. Drawn from fresh preparations.

PLATE XIV.

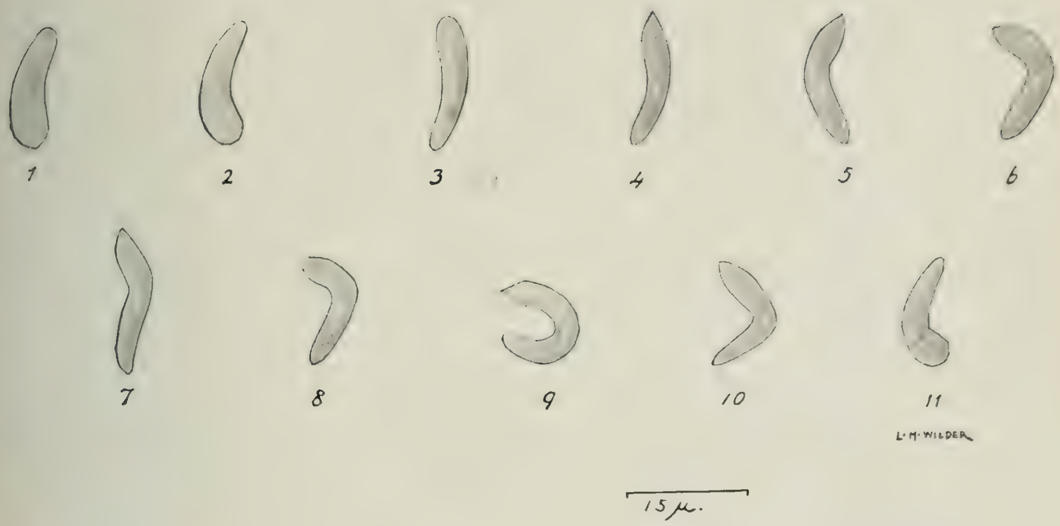


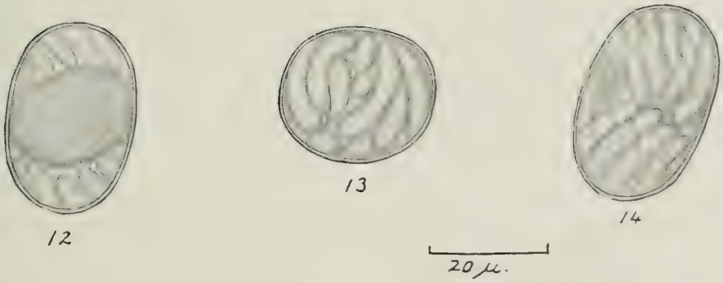
SHOWING SPORONTS IN VARIOUS STAGES, IN FRESH PREPARATIONS, MADE BY TEASING APART LIVE MITES FROM 3 TO 6 DAYS AFTER INFECTION, IN 0.3 PER CENT SALT SOLUTION.

Figs. 1-2.—Unsegmented sporonts with cyst. Figs. 3-4.—Sporonts showing budding from surface to form sporoblasts; with cyst. Drawn with camera lucida.



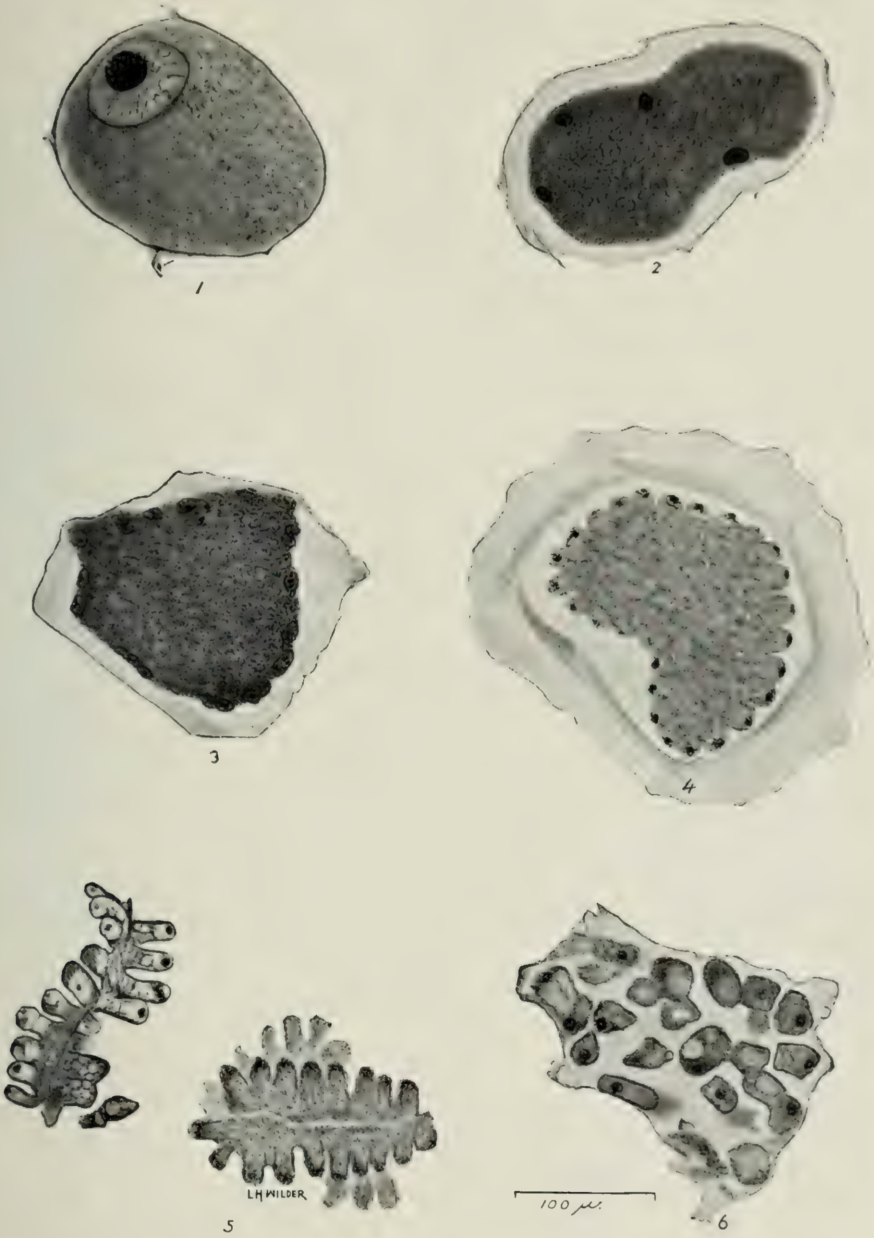
A MITE CONTAINING 3 LARGE CYSTS, WHICH HAS BEEN GENTLY CRUSHED IN SALT SOLUTION BETWEEN SLIDE AND COVER SLIP. SHOWING EXTRUSION OF A LARGE CYST CONTAINING MANY SPOROCYSTS; AND MANY FREE SPOROCYSTS. FRESH PREPARATION. SEMIDIAGRAMMATIC.





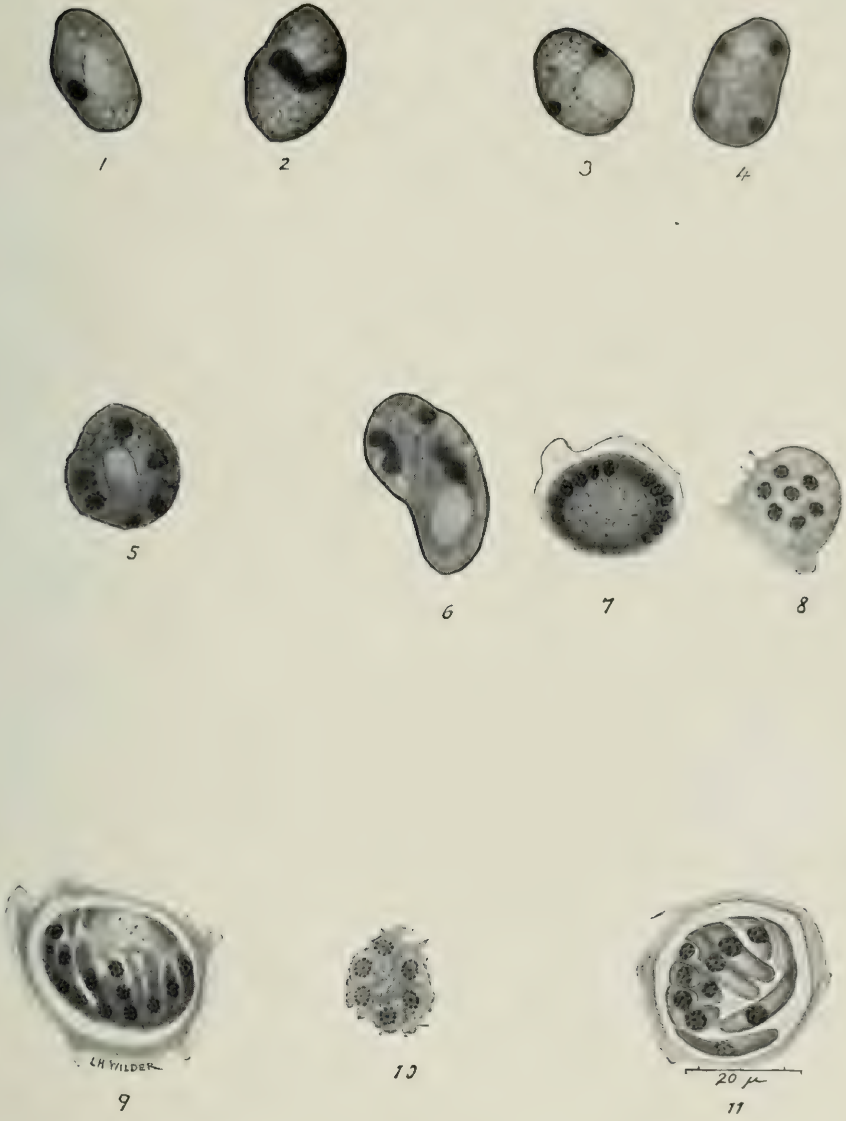
Figs. 1-11.—Free vermicules. Drawn from a fresh preparation, prepared by mixing ripe sporocysts from a crushed mite with duodenal juice obtained from a freshly killed healthy rat. Figs. 1-2.—Sporozoites unchanged. Figs. 3-11.—Sporozoites which have become active vermicules; showing striation (exaggerated) and various shapes assumed. Fig. 12.—Sporocyst from freshly crushed mite; showing central "rest" body and young sporozoites at the extremities. Figs. 13-14.—Lateral and end views of fresh sporocysts, showing completely developed sporozoites and small "rest" body.

PLATE XVII.



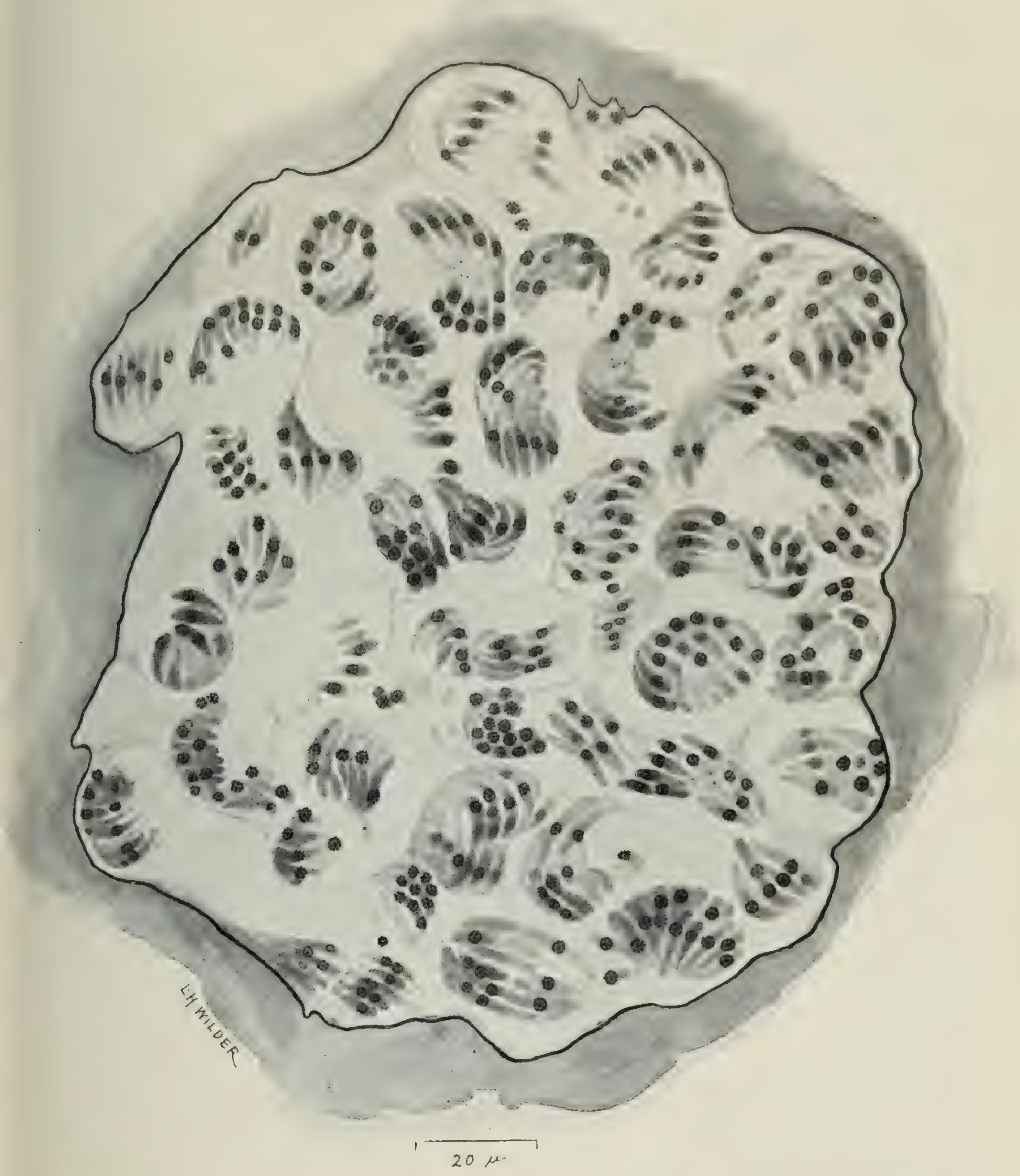
PARASITES DRAWN FROM SECTIONS OF MITES. STAINED WITH IRON HEMATOXYLIN.

Fig. 1.—Large sporont. Figs. 2-3.—Sporonts with daughter nuclei. Fig. 4.—Early bud formation. Fig. 5.—Segmentation into sporoblasts almost complete. Fig. 6.—Complete segmentation into sporoblasts. Stages shown in figs. 4, 5, and 6 are numerous in Pl. XI. Drawn with camera lucida.

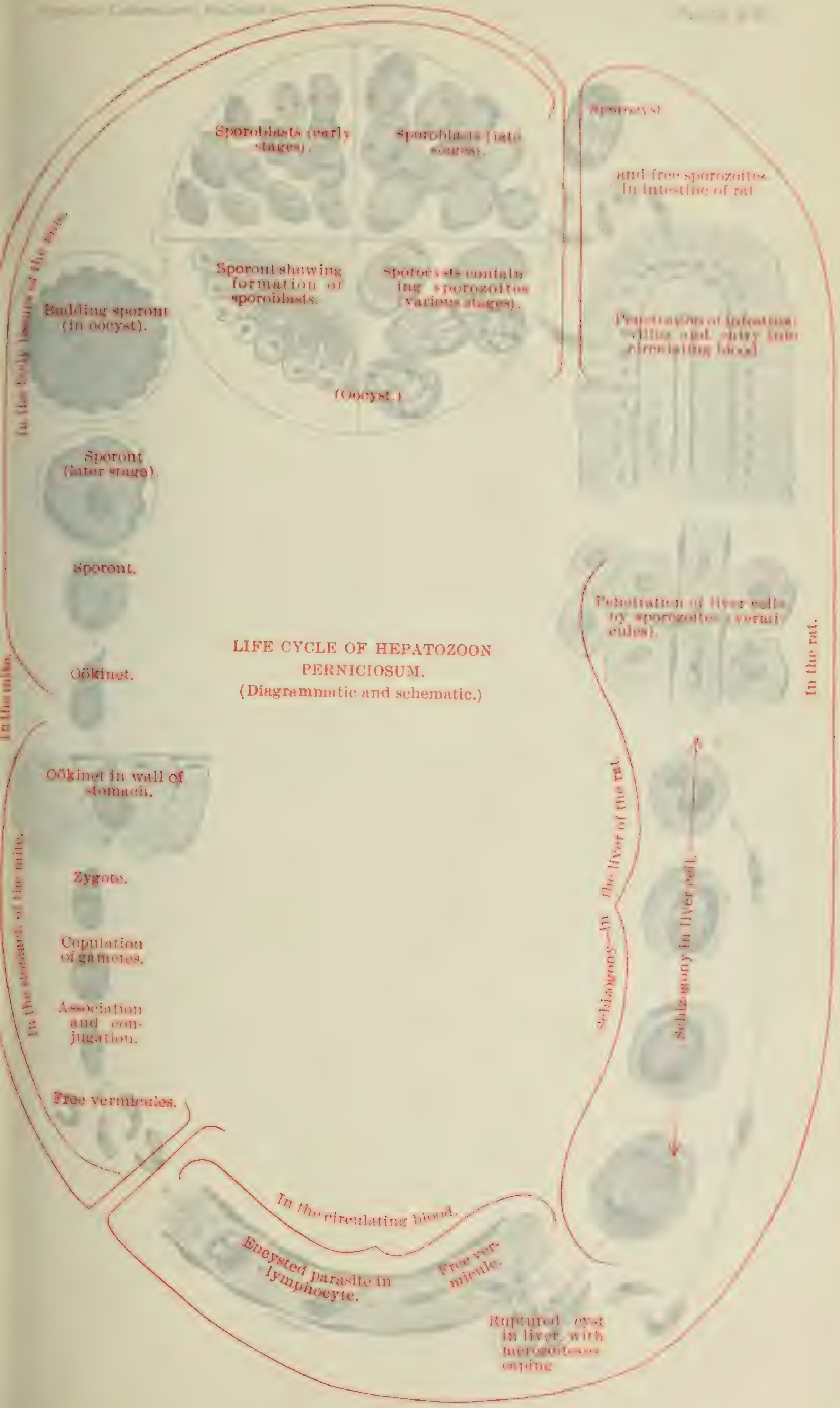


SPOROBLASTS SHOWING VARIOUS STAGES OF NUCLEAR DIVISION AND SEGMENTATION INTO SPOROZOITES; FROM SECTIONS OF INFECTED MITES, 7 TO 10 DAYS AFTER BEING PLACED UPON INFECTED RATS. STAINED WITH IRON HEMATOXYLIN.

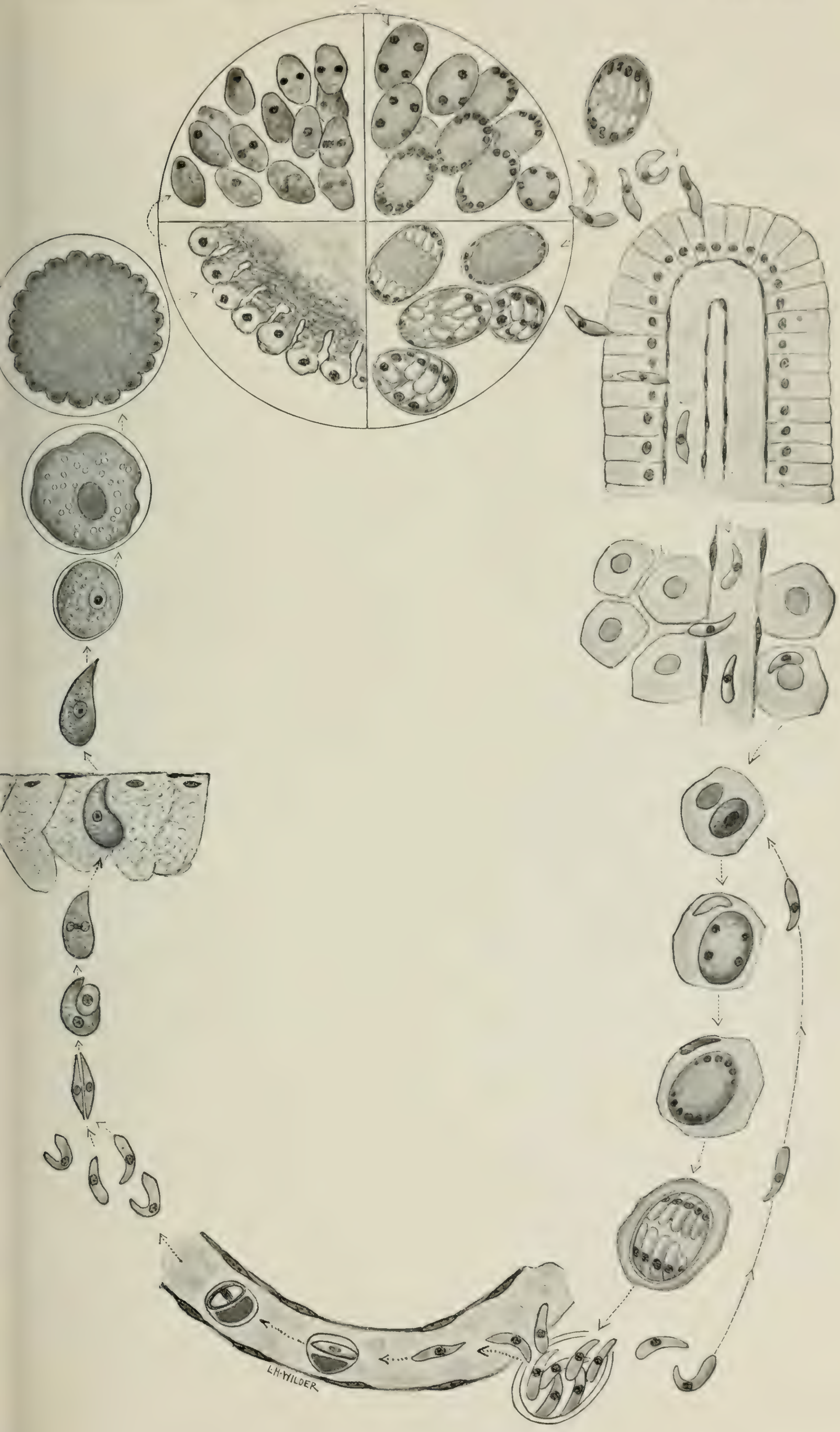
Figs. 8 and 10 show cross sections of sporoblasts near one extremity. Drawn with camera lucida.



CROSS SECTION OF LARGE OOCYST (FROM SECTION OF A MITE) CONTAINING MANY RIPE SPOROCYSTS WITH SPOROZOITES. STAINED WITH IRON HEMATOXYLIN. DRAWN WITH CAMERA LUCIDA.


Sporoblasts (early stages).
Sporoblasts (late stages).
Sporont showing formation of sporoblasts.
Sporocysts containing sporozoites (various stages).
(Oocyst.)
Budding sporont (in oocyst).
In the body tissues of the mite.
Sporont (later stage).
Sporont.
Oökinet.
In the mite.
Oökinet in wall of stomach.
Zygote.
Copulation of gametes.
Association and conjugation.
In the stomach of the mite.
Free vermicules.
In the circulating blood.
Encysted parasite in lymphocyte.
Free vermicule.
Ruptured cyst in liver, with merozoites escaping
Sporocyst
and free sporozoites In intestine of rat
Penetration of intestinal villus and entry into circulating blood
Penetration of liver cells by sporozoites (vermicules).
In the rat.
Schizogony—in the liver of the rat.
Schizogony in liver cell.

LIFE CYCLE OF HEPATOZOON PERNICIOSUM.
(Diagrammatic and schematic.)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TREASURY DEPARTMENT
Public Health and Marine-Hospital Service of the United States

HYGIENIC LABORATORY.—BULLETIN No. 47
October, 1908

STUDIES ON THYROID

I.—THE RELATION OF IODINE TO THE PHYSIOLOGICAL ACTIVITY OF THYROID PREPARATIONS

By

REID HUNT
and
ATHERTON SEIDELL

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STUDIES ON THYROID: I. THE RELATION OF IODINE TO THE PHYSIOLOGICAL ACTIVITY OF THYROID PREPARATIONS.

By Reid Hunt,
Chief, Division of Pharmacology, Hygienic Laboratory, U. S. Public Health and Marine-Hospital Service,
and
Atherton Seidell,
Chemist, Division of Pharmacology, Hygienic Laboratory, U. S. Public Health and Marine-Hospital Service.

INTRODUCTION.

Thyroid is a unique drug and occupies a place in therapeutics which can not be filled, even imperfectly, by any other known therapeutic agent. Its use was the first practical application of the doctrine of internal secretion and it still remains the most striking example of the conscious utilization, for therapeutic purposes, of an "hormone."

Although much has been learned in recent years in regard to the function of the thyroid as a glandular organ and also as to the effects of the administration of thyroid preparations upon the organism, both in health and in disease, little progress has been made in the study of thyroid as a drug. The "active principle" of the gland is unknown, and there are no generally accepted means, aside from actual clinical tests, of determining the relative value of different thyroid preparations.

A very important advance in our knowledge of thyroid as a drug was made when Baumann[a] discovered in 1895 that the gland usually contains iodine. Since that time the chief interest in the chemistry of the thyroid has centered around this iodine, but the opinions as to the physiological significance of the latter are as far apart to-day as they were twelve or thirteen years ago.

The question of the relation of the iodine to the physiological activity of thyroid is from the pharmacological standpoint the most important problem connected with this drug; this is the subject of the present bulletin.

---

[a] E. Baumann, Ztschr. f. physiol. chem., Strassb., 1895–96, **21**, p. 319.

Before considering the main problem, however, a few words may be devoted to certain phases of the older history of the thyroid. The relation of iodine to this gland had been for many years a matter of interest to physicians and was much discussed even before Baumann's important discovery. It has long been recognized that iodine has some special relation to the thyroid. Seven years after the discovery of the element iodine by Courtois, in 1812, Straub, of Berne, suggested that it was the active principle of the "toasted sponges" (Spongia "usta" or "tosta") and the "Aethiops vegetabilis," which had been used in the treatment of goiter at least since the thirteenth century.[a] Since 1820 iodine, as such or in combination, has been in constant use in the treatment of diseases of the thyroid.

About the middle of the last century great interest was aroused by the work of Chatin, Marchand, and others on the relation of iodine in drinking water to goiter. The more recent history of this subject began with the revival of interest in the physiology and pathology of the thyroid gland which was stimulated by the work of Gull and Ord on myxoedema and of Kocher and Reverdin on the effects of the removal of the thyroid in man. This work was followed, partly as a result of the stimulus of Brown-Sequard's ideas concerning internal secretion, by the introduction of organotherapy in the treatment of diseases of the thyroid. The brilliant results of this form of treatment led to the search for an "active principle," and this work culminated in the discovery of iodine in the thyroid by Baumann. In the year of Baumann's discovery Kocher,[b] who had been much impressed by the similarity of the effects of iodine and thyroid in the treatment of goiter, had suggested the desirability of examining the thyroid for iodine; the tests made in Berne were, however, negative.

Baumann at first considered the iodine containing substance which he isolated and called iodothyrine to be the active principle of the thyroid; although he recognized later that in the gland much of this iodothyrine is in combination with proteins, neither he nor his coworkers seemed to have entertained any doubt that the iodine was very important for the physiological activity of the thyroid.

Following Baumann's discovery, determinations of the amount of iodine were made in the thyroid in a great variety of conditions; the influence of health, disease, sex, pregnancy, age, locality (near or remote from the sea, on the plains, and in the mountains), diet, climate, etc., was investigated. The amount of iodine in the thyroids of a great variety of animals was also determined. Far-reaching conclusions were drawn from such studies, especially as regards the relation of iodine to the diseases of the thyroid itself (various forms of goiter) and to various forms of insanity. A vast number of data were collected, but these were often contradictory and not easily correlated.

---

[a] *cf.* E. Harnack, München. med. Wchnschr., 1896, **43,** p. 196; G. Monéry, Fonction iodeé de la glande thyroide, Thesis, Lyons, 1903; Th. Kocher, Die Therapie des Kropfes, Deutsche Klinik, Berl. and Wien, 1905, **8,** p. 1115.

[b] Th. Kocher, Cor. Bl. f. schweiz. Aerzte, 1895, **25,** p. 3.

The result was that within a few months after Baumann's first publication on the subject objections began to be urged against the view that the iodine is an important constituent of the thyroid. These objections have been repeated and still others urged up to the present time; they will be considered in detail in the following pages.

Part I.

THE PARALLELISM BETWEEN THE PHYSIOLOGICAL ACTIVITY AND THE IODINE CONTENTS OF THYROID.

A. HISTORICAL.

The views as to the relation of the iodine to the physiological activty of the thyroid may be classified under three heads: (1) Some nvestigators hold that the activity of thyroid is directly proportional to and dependent upon its iodine content and that thyroid free of iodine has no physiological activity. (2) There is another group of writers who take the view that there is no relation between the physioogical activity of thyroid and its iodine content and that no importance should be attached to the iodine which is usually present in the thyroid. (3) Other writers admit that there is a parallelism between the physiological activity and the iodine content, but deny that the former is dependent upon the latter. Some of those holding this view apparently consider that the iodine is simply associated with the active part of the gland in some unknown, perhaps accidental, way. Others hold that the larger amount of iodine in active thyroid results from the activity of the gland itself; they consider that the more active the gland the larger the amount of iodine it is able to hold in combination. This latter view is closely connected with the theory that one of the functions of the thyroid is to take up injurious substances (including iodine) and to render them harmless.

Some of these theories have been combined. Thus von Cyon supposes the thyroid to take up iodine, by which process the latter is rendered harmless and at the same time a new compound, useful as an internal secretion, is formed.

We believe that these conflicting views have resulted partly from the failure of previous writers to recognize that thyroid free of iodine has a certain degree of physiological activity and partly from the fact that there has been a confusion of several distinct problems and that authors have often looked upon the subject from but a single point of view.

Baumann and some of the other earlier workers in this field apparently entertained no doubts as to the iodine being an important part of the "active principle" of the thyroid. They based this view upon the physiological action of iodothyrine in health and disease[a] and on the fact that iodine is so generally found in the thyroid in health and is apparently absent or present in diminished amounts in certain forms of goiter.

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[a] E. Baumann and E. Roos, Ztschr. f. physiol. Chem., Strassb., 1895–6, **21**, p. 487.

Before discussing in detail the chief arguments in favor of Baumann's view certain of the objections which have been made to it may be considered. The principle of these objections may be classified as follows:

a. Iodine is not invariably found in the thyroids of healthy animals; when it does occur there are great variations in its amount.

b. Artificially iodized proteins, including those of the thyroid itself, are inactive physiologically or the activity is not at all proportional to the amount of iodine which has been added; this is also true of iodothyrine to which iodine has been added *in vitro.*

c. Thymus was reported to be of value in the treatment of goiter.

d. The reputed "active principle" of the thyroid (Baumann's iodothyrine) did not prevent tetany in animals from which the thyroids had been removed.

e. Efficiency of iodine compounds other than iodothyrine in goiter.

These points may now be considered in more detail:

a. Iodine is not invariably present in the thyroid.—Miwa and Stoeltzner[a] early laid great emphasis on this point. They argued that if the theory that iodine is an essential constituent of the thyroid is correct, then the absence of iodine in the thyroid of an individual would have the same serious effects as the absence of the gland itself. They pointed out that Baumann had reported the frequent absence of iodine from the thyroid of children, and of dogs fed on meat; yet neither the children nor the dogs had shown any indications of thyroid insufficiency. Miwa and Stoeltzner confirmed Baumann's observations on the frequent absence of iodine from the thyroids of children; they also stated that the hen's egg contains no iodine and that the young chick thus begins life without iodine. They emphasized the dependence of the iodine content of the thyroid upon the diet. From these facts Miwa and Stoeltzner argued that the iodine usually found in the thyroid has no more significance than the traces of copper so often found in the liver. Similar arguments were advanced with great emphasis by Neumeister [b] at about the same time; he stated that the iodine of the thyroid has absolutely no physiological significance.

These arguments of Miwa and Stoeltzner and of Neumeister have been repeated to the present time; the facts upon which they were based have received abundant confirmation.[c] Thus Töpfer in Vienna found no iodine in the thyroids of cattle.[a] Roos[b] found no iodine in the thyroids of three foxes; none in that of a polecat; none in that of a wild cat; he found it in but two of six martens; it was absent from the thyroids of four domestic cats and present in but traces, or in very small amounts, in those of five others; it was absent in four of eleven dogs, in two of four horses, and three of seven hogs.

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[a] S. Miwa and W. Stoeltzner, Jahrb. f. Kinderh., 1897, **45,** p. 83.

[b] R. Neumeister, Lehrbuch der physiologischen Chemie, 2d ed., 1897, p. 520. Cf. also Halliburton in Schäfer's Textbook of Physiology, 1898, **1,** p. 89.

[c] An abstract of the literature showing how frequently iodine is absent from the thyroids of infants is given on page 33 of this bulletin.

In many cases the presence or absence of iodine is dependent upon the character of the food which the animal has received.

The thyroids of herbivorous animals, whose food usually contains small amounts of iodine, are almost always richer in this element than are those of carnivorous animals, whose food is usually poor in iodine.[c] Sheep pastured near the sea may have double the amount of iodine in their thyroid as those pastured in inland regions.[d] The amount of iodine in the thyroid of omnivorous animals (hog, dog, man) is especially variable. Nagel and Roos[e] found the percentage in the dried glands of hogs to vary from 0 to 0.075, although the glands were obtained from the same slaughterhouse.[f] Baumann found the percentage of iodine in the thyroid of horses to vary from 0.06 to 0.17.[g]

As a rule it is not possible to detect any difference between the animals which have a large percentage of iodine in their thyroids and those which contain none or only traces; removal of the gland is followed by as severe symptoms in the latter as in the former. Thyroid free of iodine seems to meet the needs of the body as well as the thyroid that contains iodine.[h]

Human thyroids also contain quite variable amounts of iodine, as was first pointed out by Baumann.[i] Jolin[j] has recently summarized the entire literature on the occurrence of iodine in the thyroid of man and has reported a very large number of analyses of such thyroids obtained in Sweden; he found great variations in the percentage of iodine and was unable to discover any constant relation between its presence or amount and conditions of health or disease. He asks, "Can not the great variations in the iodine content of not only pathologically altered thyroids but also of normal glands, and especially the fact that thyroids may be found in adults as well as in children which contain no iodine or scarcely traces of it, simply depend upon the fact that the iodine is an accidental constituent

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[a] Töpfer, Wien. klin. Wchnschr., 1896, **9**, p. 141.

[b] E. Roos, Ztschr. f. physiol. Chem., Strassb., 1899, **28**, p. 55.

[c] E. Roos, l. c.

[d] Th. Suiffet, J. de pharm. et chim., Par., 1900, [6] **12**, p. 50.

[e] W. G. Nagel and E. Roos, Arch. f. (Anat. und) Physiol., Suppl.-Bd., 1902, p. 267.

[f] A specimen of hog thyroid examined by us contained 0.33 per cent iodine.

[g] Baumann, Ztschr. f. physiol. Chemie, Strassb., 1896, **22**, p. 17.

[h] Exceptions to this will be referred to later.

[i] E. Baumann, Ztschr. f. physiol. Chem., Strassb., 1896, **22**, p. 1.

[j] S. Jolin, Festschrift, O. Hammarsten, Upsala, 1906.

(nebensächlicher Bestandteil) of the thyroid and its secretion?" He further states that the fact that in the body iodine is found chiefly in the thyroid "does not prove that the iodine is necessary for the activity of this gland; it may be explained by supposing that the thyroid has, among other functions, that of taking up an excess of iodine in the blood and storing it."

Several other recent writers have expressed somewhat similar views.[a] This point of view is, however, by no means new; Roos[b] referred to it in 1899 in the following words:

> To consider the accumulation of iodine in the thyroid as an accidental process which has no greater significance for the body than the storing up of certain metals in the liver seems to me to be a very unsatisfactory conception; for on it it is entirely unintelligible why the thyroid should use the iodine for the making of a substance (iodothyrine) which has such a marked effect upon metabolism and myxoedema instead of combining it in a much less active form, as occurs when iodine is allowed to react with proteins outside of the body.

Such studies as the above (they may be called statistical studies) have undoubtedly brought to light many facts, some of which are difficult to satisfactorily explain. Still they do not seem to us to weaken the conclusion that iodine is an important constituent of the thyroid. All the writers who have discussed this subject seem to have taken one of two extreme views—that iodine is necessary to any physiological activity, or that it has no part at all in rendering the thyroid active. No one seems to have suggested an intermediate view, viz, that thyroid free of iodine may have a certain degree of activity, although this is much less than that of thyroid containing iodine, until one of the present writers discovered that it is distinctly active in altering the resistance of animals to certain poisons.[c] This subject will be discussed in detail in the experimental part of this section. It will suffice for the present to state that we believe this conception and the conclusions that may be drawn from it go far toward answering the arguments for the unimportance of iodine which have been drawn from statistical studies.

b. Physiological inactivity of artificially iodized proteins and colloid.—The question of the relation of the iodine to the activity of the thyroid was approached experimentally by Hutchinson,[d] who prepared an artificial iodized nucleo-albumin from the thymus of calves. Samples of it contained from 4 to 7 per cent of iodine; it had no effect in myxoedema or upon the pulse, temperature, or weight of subjects to whom it was administered. Hellin[e] had previously found iodized albumin and nucleo-albumin prepared from the spleen to be inactive. Blum[a] had also found iodized albumin almost without effect upon metabolism. Hutchinson also increased artificially the percentage of iodine in colloid derived from the thyroid. Although such a product contained nearly ten times as much iodine as the original material, it had no greater physiological activity. Hutchinson drew the following conclusions from these results:

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[a] Bunge, Lehrbuch der Physiol. des Mensch., 2d ed., 1905, **2,** p. 631; S. J. Meltzer, N. Yorker med. Monatschr., 1907, **19,** p. 223; Abderhalden, Lehrbuch der physiol. Chem., 1907, p. 647.

[b] E. Roos, Ztschr. f. physiol. Chem., Strassb., 1899, **28,** p. 59.

[c] R. Hunt, J. Am. M. Ass., 1907, **49,** p. 1325.

[d] R. Hutchinson, J. Physiol., 1898–99, **23,** p. 181.

[e] D. Hellin, Arch. f. exper. Path. u. Pharmakol., Leipzig, 1897, **40,** p. 121.

> One would conclude from the whole evidence that the iodine in the thyroid gland, if it plays an essential part in the activity of the latter at all, does so simply in virtue of the special form of combination in which it is present.[b]

Hutchinson had apparently concluded from earlier work that not much importance was to be attached to iodine as a factor in determining the activity of the thyroid. Thus in 1896 he wrote as follows:[c]

> It must not * * * be supposed * * * that the iodine is necessarily the essential factor in the activity of the thyroid gland. One has only to realize the small proportion in which it is normally present—only a few milligrams in a whole gland—to be convinced of that. * * * Baumann has himself failed to find any iodine at all in the thyroid of some children. Yet I suppose such a gland is none the less active.

Blum[d] and Roos[e] had also early pointed out that thyroid proteins or iodothyrine to which iodine had been added *in vitro* were physiologically inactive or less active than the natural products containing much less iodine. Blum used this observation as an argument against the view that the iodine is a factor in the physiological activity (or "toxicity," as Blum denies that the thyroid produces an internal secretion) of the proteins of the thyroid; he applied this view also to the iodine which has been taken up by the living gland, maintaining that the iodization *in vivo* is strictly analogous to that *in vitro*. Roos[f] endeavored to disprove Blum's theory by feeding iodine to dogs until the iodine content of the thyroid had been considerably increased; he stated that such thyroids showed increased physiological activity. Blum[g] points out that Roos's figures are hardly conclusive in this respect. We have recently studied this phase of the subject, using new methods for determining the activity of the thyroid, and have completely confirmed Roos's results. (See Part II of this bulletin.)

c. *The value of thymus in goiter.*—Further arguments against the view that the iodine is an important constituent of thyroid have been based upon the statements that thymus has an effect in goiter similar to that of thyroid.[a] Since most writers have found either no iodine in the thymus or only traces (in which case it was usually attributed to admixture with thyroid[b]) the conclusion has been drawn that thymus free of iodine has a physiological effect similar to that of thyroid. In reply to this it may be stated that later writers seem to be agreed that thymus has very little activity in goiter. Even granting that thymus does possess a certain degree of activity of a character similar to that of the thyroid, this would not prove that iodine is not a very important constituent of the thyroid. One of the present writers[c] recently showed that there is present in several organs of the body a substance or substances having a physiological action similar, at least in some respects, to that of thyroid, but that the latter, apparently on account of its iodine content, is far more active.

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[a] F. Blum, München. med. Wchnschr., 1898, **45**, p. 270.

[b] Similar views have been expressed by G. Reinbach, Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1898, **3**, p. 309, and L. B. Mendel, Am. J. Physiol., Bost., 1900, **3**, p. 290.

[c] R. Hutchinson, J. Physiol., 1896, **20**, p. 494.

[d] F. Blum, München. med. Wchnschr., 1898, **45**, p. 335.

[e] E. Roos, Ztschr. f. physiol. Chem., Strassb., 1898, **25**, p. 242.

[f] E. Roos, Ztschr. f. physiol. Chem., Strassb., 1899, **28**, p. 40.

[g] F. Blum, Verhandl. des Kong. f. inn. Med., 1906, **23**, p. 196.

d. Inefficiency of iodothyrine in tetany.—Another argument against the view that the iodine-containing substance (iodothyrine) obtained from the thyroid by Baumann is the active constituent of the thyroid was that it did not prevent tetany in thyroidectomized animals.[d] These experiments were performed before the importance of the parathyroids was recognized and the investigators were endeavoring to control symptoms with which the thyroid had little or nothing to do.

e. Efficiency of iodine compounds other than iodothyrine in goiter.—Baumann argued that iodothyrine was the active principle of the thyroid from the fact that its administration produced the same effects upon certain forms of goiter as did that of the thyroid itself. But attention was called to the fact[e] that a great variety of iodine compounds, both inorganic and organic, had a similar effect in these conditions. Evidently, however, such arguments do not bear directly upon the question whether the iodine is of importance to the thyroid. Recent work indicates that these different forms of iodine are efficient simply because the iodine has a special affinity for the thyroid. The effect of iodine in the treatment of goiter is in reality an argument in favor of the view of the importance of iodine in the thyroid.

A very little consideration suffices to show that none of the above arguments have any very direct bearing upon the problem with which we are concerned, viz, the use of thyroid as a drug. It is not the condition of the thyroid in a living animal which is of interest in this connection. There may be and probably are delicate mechanisms by which the amount and strength of the secretion given off by the thyroid is accurately adjusted to the needs of the body. A gland may be supposed to form a large amount of very active material, but this may not reach the circulation owing to some abnormalities in the blood or lymph vessels or possibly to the absence of some stimulus which normally causes the secretion of the material. In the body the thyroid which has undergone cystic degeneration may be very neffective in preserving health; but removed from the body and administered as a drug it may have pronounced effects. Similarly we may suppose that in some cases of Grave's disease the thyroid contains normal amounts of active material but that the conditions for the rapid secretion or for the reabsorption of the iodine are so favorable, or that the capacity of the organism to destroy the secretion is lowered, so that there is an accumulation of the active material in the body outside of the thyroid. In such a case there might be a condition of hyperthyroidism, but the gland when administered to an animal as a drug might not be very active. In other words, he effects of a dead organ when administered as a drug can not be compared with the activities of the living organ in the body. Many of the writers who have discussed the relation of iodine to the thyroid have confused these two distinct problems.

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[a] J. Mikulicz, Berl. klin. Wchnschr., 1895, **32**, p. 342; G. Reinbach, Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1898, **3**, p. 309; R. H. Cunningham, J. Exper. Med., N. Y., 1896, **3**, p. 227.

[b] E. Baumann, München. med. Wchnschr., 1896, **43**, p. 311; R. H. Cunningham, l. c.; H. G. Wells, J. Am. M. Ass., Chicago, 1897, **29**, p. 1009; L. B. Mendel, Am. J. Physiol., Boston, 1900, **3**, p. 285.

[c] R. Hunt, J. Am. M. Ass., Chicago, 1907, **49**, p. 1325.

[d] *cf.* G. von Bunge, Lehrbuch der Physiologie des Menschen, 2 ed., 1905, **2**, p. 632; E. Roos, Ztschr. f. physiol. Chem., Strassb., 1899, **28**, p. 41; 1899, **27**, p. 37.

[e] *cf.* Von Bunge, l. c.

If we now turn to the experiments in which the question of the relation of iodine to the thyroid when used as a drug has been approached directly, we find that there is no difference of opinion: all who have worked upon the problem from this standpoint agree as to he importance of the iodine. Unfortunately, however, the experiments of this character are very few and the results are not quantitative.

The experiments bearing on this phase of the subject may be divided into three groups—(*a*) those in which the action of the thyroid upon metabolism, (*b*) upon different forms of goiter, and (*c*) upon he circulation was investigated.

The most important work upon the relation between the iodine content of the thyroid and its effects upon metabolism was done by Roos,[a] who recorded the results of three such experiments upon a dog. In the first experiment the administration of 5 grams of desiccated children's thyroid containing 0.025 per cent iodine had almost no effect upon the excretion of nitrogen or upon body weight; later he administration of 5 grams of children's thyroid containing 0.18 per cent iodine caused an increase in the excretion of nitrogen of about 10 per cent. In the second experiment also a greater effect was produced by the administration of the thyroid containing the larger percentage of iodine. In the third experiment, in which the dried thyroids of dogs were administered, 5 grams of a preparation containing no iodine had no effect, whereas 5 grams of a preparation which contained 0.35 per cent iodine was followed by a distinct increase in the nitrogen excretion and a slight loss of weight.

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[a] E. Roos, Ztschr. f. physiol. Chem., 1899, 28, p. 40.

Roos's experiments were very few in number and the results not very striking, and perhaps do not justify the very positive conclusions he drew from them.[a] Still they point very distinctly to the conclusion that thyroid rich in iodine has a more marked effect upon nitrogen metabolism than does thyroid poor in, or free of, iodine.

Roos also describes the effects of desiccated hog thyroids containing different percentages of iodine upon four cases of parenchymatous goiter. In all cases thyroid free of iodine had a very slight or doubtful effect, whereas thyroid containing iodine had a marked action in reducing the size of the goiter. In one case the use of doses of 0.5 gram thyroid containing 0.008 per cent iodine had a distinct effect, but the effect of the administration of equal doses of a thyroid containing 0.08 per cent iodine was more marked.

Roos himself recognized that only an approximate estimation of the quantitative effects of thyroid was possible either in metabolism experiments or in the treatment of goiter. He also pointed out the difficulties of drawing any conclusions from observations upon thyroid-ectomized animals, and stated that he deemed it useless to try this method.

Oswald tested the activity of thyreoglobulin containing little iodine upon metabolism, and von Cyon investigated its effects upon the irritability of the vagi.[b] In the two experiments upon metabolism reported by Oswald the thyreoglobulin poor in iodine produced no effect in one case and very slight, if any, in the other; thyreoglobulin rich in iodine produced a marked increase in the nitrogen excretion. Von Cyon reported that thyreoglobulin obtained from colloid goiters and which contained 0.19 per cent iodine had a distinct effect upon the irritability of the vagi, but that this was less than that produced by normal thyroid with a higher percentage of iodine. Von Cyon and Oswald[c] had already found thyreoglobulin free of iodine obtained from goitrous calves to be without any effect upon the circulation. Oswald further states that thyreoglobulin from hog thyroid containing 0.5 per cent iodine seemed more active than that of the human thyroid with 0.3 per cent iodine.[d]

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[a] *cf.* F. Blum, Verhandl. d. Kong. f. inn. Med., 1906, **23**, p. 196.

[b] A. Oswald, Arch. f. path. Anat. [etc.], Berl., 1902, **169**, p. 461; Beitr. z. chem Physiol. u. Path., Brnschwg., 1902, **2**, p. 555.

[c] E. von Cyon and A. Oswald, Archiv. f. d. ges. Physiol., Bonn, 1901, **83**, p. 202.

[d] A. Oswald, Beitr. z. chem. Physiol. u. Path., Brnschwg., 1902, **2**, p. 556 (footnote)

Marine and Williams[a] have recently reported two experiments on he effects of feeding desiccated sheep thyroid containing different ercentages of iodine to dogs. Of one preparation which contained .0292 per cent iodine 11 grams fed to a dog in eighteen days did not ause a loss of weight, and the fresh thyroid of the dog on analysis ielded but 0.173 milligram iodine per gram. The second preparaion had 0.1092 per cent iodine. The dog which received 11 grams f this in eighteen days lost 454 grams weight and its fresh thyroid ontained, per gram, 0.439 milligram iodine.

These experiments, although very few in number and not of a haracter to admit of quantitative results, indicate clearly that there s a relation between the iodine content and the physiological activity f thyroid preparations.

B. EXPERIMENTAL.

METHODS.

None of the methods discussed in the above review are suitable r a quantitative study of the physiological effects of different hyroid preparations. They are all time-consuming, and so do not dmit of many experiments; furthermore, they are not adapted for iowing small differences in activity. They did not suffice, for xample, to demonstrate the physiological activity of thyroid free f iodine. It may also be mentioned that different animals and ifferent patients may react differently to the same preparation;[b] iis has been observed both clinically and experimentally. If it ere possible to test the various thyroid preparations upon the same idividual fairly concordant results could probably be obtained, but ich experiments are, as a rule, not feasible.

Before beginning the description of the method which forms the asis of the experiments described in this bulletin a few words may e said in regard to some observations which we have made upon the lation between the activity of thyroid preparations and the loss body weight which their ingestion may produce. Many of the sults are given in the protocols of the experiments which follow, ut in addition to these several experiments were performed expressly r determining whether a method based upon the loss in weight of e animals could be used for estimating the relative activity of thyroid preparations. The figures given in the protocols show the loss of weight for relatively short periods (ten to twenty days) and do not indicate that the method is of practical application. In the special experiments which were undertaken the animals (rats and guinea pigs) were fed during longer periods or until death occurred. The results showed that in general the thyroid with the largest percentage of iodine produced death earliest, but in no case was more than a rough quantitative application of the data possible. It is probable that the primary effect of thyroid feeding is the same in different animals, but that other factors, such as appetite, rate of excretion, etc., which can not be controlled, are involved, and these determine the final result.

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[a] D. Marine and W. W. Williams, Archives of Internal Medicine, 1908, **1**, p. 378.

[b] The fact that there are such variations in the susceptibility of different individuals n not be considered an argument against the desirability of having thyroid preparans of known strength. It is especially important that when the susceptibility of a tient to a given drug has been determined that treatment should be continued with preparation of known strength—not with one half or three times as strong, as may adily occur with present commercial thyroid preparations.

The method which we have developed for comparing the physiological activity of different thyroid preparations has for its basis the effect of thyroid feeding upon the resistance of animals to certain poisons. Having found that the administration of thyroid alters the resistance of animals to certain poisons, we performed a large number of experiments in order to determine which poisons were most suitable for comparative studies. The principal poisons tested on mice are included in the following list, and in most cases, as will be seen, negative results were obtained. With many of the compounds in the list but two or three experiments were performed; hence we can not state positively that the feeding of thyroid in these cases had *no* effect upon the resistance to the substance in question. The thyroid, in amounts varying from 0.001 to 0.1 gram per day, was fed in the form of cakes; the poisons were injected subcutaneously

Acetonecyanhydrine	Resistance slightly increased (?).
Acetonitrile	Resistance much increased.
Acetylmorphine ("Heroin")	Resistance diminished.
Antipyrine	No effect.
Atropine	No effect.
Benzonitrile	No effect.
Butylcarbylamine (tertiary)	No effect.
Caffeine	No effect.
Capronitrile	No effect.
Chloral hydrate	Resistance diminished(?).
Codeine	Resistance diminished.
Ethylcarbylamine	No effect.
Ethylenecyanide	Resistance increased.
Guanidine carbonate	No effect.
Guanidine sulphocyanate	No effect.
Hydrocyanic acid	No effect.
Isovaleraldehydecyanhydrine	No effect.
Methylamine	No effect.
Methylethylketonecyanhydrine	No effect.
Morphine	Resistance diminished.
Nicotine	No effect.

Nitroprussiate of soda	No effect.
Ox gall	No effect.
Phenol	No effect.
Picrotoxin	No effect.
Sodium arsenate	No effect.
Sodium oxalate	No effect.
Strychnine	No effect.
Trimethylenecyanide	Resistance increased.

The poisons to which the resistance of mice was most markedly ltered by the feeding of thyroid were acetonitrile and morphine. ince the susceptibility of rats and guinea pigs to these poisons was lso changed by the administration of thyroid, they were selected for omparative studies.

The advantages of a method of this kind are that the experiments re quickly and easily carried out and that they admit of the detecion of smaller differences in activity than do the methods hitherto mployed. It is not possible to state positively that the physiological ctivity determined in this manner is in all respects a true index of he therapeutic efficiency of the thyroid, but there are many reasons or believing this to be the case. In the first place, thyroid is preminently a drug that influences metabolism, and the changes in the esistance of animals to the poisons here considered depend upon eep-seated, although little understood, changes in metabolism. In he second place, the results obtained are in complete accord with hose observed in clinical studies and with those obtained by other hysiological methods. In any case there seems to be much reason or supposing that these reactions are as true an index of the real hysiological activity of thyroid as is its effect upon the excretion of rea or upon the circulation.

The experiments were performed upon mice, rats, and guinea pigs. he thyroid was fed to guinea pigs in the form of tablets; to mice nd rats in the form of cakes, according to Ehrlich's method. Each ake weighed about 4 grams; mice ate about one cake, rats from one nd a half to three cakes per day. The poisons were dissolved in ater and injected subcutaneously.

I. EXPERIMENTS WITH ACETONITRILE.

Acetonitrile or methylcyanide, $CH_3 CN$, seems to produce toxic ffects chiefly, if not entirely, through the slow liberation in the body f hydrocyanic acid. The views as to the manner in which this ormation of hydrocyanic acid takes place have been discussed in a revious bulletin from this laboratory.[a] An additional argument for he view that the toxic effects of acetonitrile are due to the formation of hydrocyanic acid may perhaps be found in the comparison of the effects of these two poisons upon sulphur metabolism. In the publication referred to above it was stated that under the influence of acetonitrile the "percentage of sulphur excreted as neutral sulphur frequently rose from 25 to 65 or more; the total excretion was not much changed, but the oxidized sulphur frequently almost disappeared." Results very similar to the above have recently been obtained by Richards and Wallace,[a] and by Loewy, Wolf, and Oesterberg[b] with potassium cyanide and hydrocyanic acid.

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[a] Bulletin 33: Studies in Experimental Alcoholism, 1907, p. 9.

Richards and Wallace found in one experiment upon a dog poisoned with potassium cyanide that while the total amount of sulphur eliminated remained unchanged the percentage eliminated in the oxidized state was 64.53 on the normal day and 48.78 on the day of poisoning. Loewy, Wolf, and Oesterberg found that in severe poisoning by hydrocyanic acid the sulphur eliminated as neutral sulphur increased from 28.6 per cent to 54.8 per cent. The greater effect upon the sulphur metabolism observed in our experiments was probably due to a more prolonged action of the hydrocyanic acid.

As has been already stated, the administration of thyroid causes marked changes in the susceptibility of animals to acetonitrile. Since thyroid does not alter the resistance of animals (or at least of mice) to hydrocyanic acid,[c] it may be concluded that its action is exerted chiefly upon the processes by which acetonitrile is decomposed in the body and not upon the hydrocyanic acid which is formed from it. In fact, acetonitrile seems to be a very delicate test for detecting changes in metabolism brought about not only by the thyroid and certain other organ products,[d] but by different diets,[e] inanition, etc.

a EXPERIMENTS ON MICE.

Preliminary experiments and theoretical considerations.—When small amounts of thyroid are fed to mice for a few days these animals acquire a markedly increased resistance to acetonitrile. This is true not only for white but also for wild gray mice, although most of the experiments were performed upon the former, and unless otherwise stated, white mice are referred to in all of the following experiments.

The following results may be quoted to illustrate from what large amounts of acetonitrile mice which have received small amounts of thyroid may recover. Only those experiments are quoted which show the maximum non-fatal and the minimum fatal dose:

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[a] A. N. Richards and G. B. Wallace, J. Biol. Chem., 1908, **4**, p. 179.

[b] A. Loewy, C. G. L. Wolf, and E. Oesterberg, Biochem. Ztschr., 1908, **8**, p. 132.

[c] R. Hunt, J. Biol. Chem., 1906, **1**, p. 42.

[d] J. Am. M. Ass., 1907, **49**, p. 1325.

[e] Proc. Soc. Exper. Biol. and Med., 1906, **3**, p. 15. These results will soon be published in detail.

SERIES I.

A. SHEEP THYROID; 0.179 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1908.		
February 1.....	22.62	Feeding of cakes each containing 0.002 gm. thyroid begun.
4.....	23.02	
7.....	22.69	
9.....	23.35	Acetonitrile, 105.08 mgms., i. e., 4.5 mgms. per gm. mouse. Survived.
February 1.....	22.56	Thyroid as above.
4.....	22.18	
7.....	20.62	
10.....	20.28	Acetonitrile, 93.29 mgm., i. e., 4.6 mgms. per gm. mouse. Died 2 hours.

B. CONTROLS.

Date.	Weight of mouse.	Remarks.
1908.		
February 1....	18.83	Feeding of cakes without thyroid begun.
4....	20.01	
7....	20.19	
10....	19.02	Acetonitrile, 4.76 mgms., i. e., 0.25 mgm. per gm. mouse. Survived.
February 1....	16.49	Cakes as above.
4....	15.56	
7....	15.79	
10....	16.31	Acetonitrile, 4.24 mgms., i. e., 0.26 mgm. per gm. mouse. Died 10 hours.

Summary.—The results of these experiments may be summarized as follows:

	Gm. thyroid fed daily.	Mgm. I in thyroid.	Weight of mouse in gms.	Amount of acetonitrile in mgms.		Result.
				Per mouse.	Per gm. mouse.	
A..........................	0.002	0.0036	23.35	105.08	4.5	Survived.
	.002	.0036	20.28	93.29	4.6	Died.
B..........................	0	0	19.02	4.76	.25	Survived.
	0	0	16.31	4.24	.26	Died.

Thus a mouse (A) which had received thyroid recovered from seventeen times the relative amount of acetonitrile fatal to the control. Since 0.25 mgm. per gram is the maximum amount of the nitrile from which a control (B) recovered, we are justified in assuming that this represents the maximum amount of nitrile which the mice of this series could, under normal conditions, neutralize; for a mouse weighing 23.35 gms. this would be a total of 5.84 mgms. The mouse which weighed 23.35 gms. and which had received thyroid recovered, however, from 105.08 mgms. Hence we may conclude that the thyroid had enabled the mouse to neutralize, or resist in some way, 99.24 mgms. acetonitrile. The mice seldom ate an entire cake (containing 0.002 gm. thyroid) in a day; hence they had received less than 20 mgms. thyroid in all.

SERIES II.

A. DOG THYROID; 0.111 PER CENT IODINE

Date.	Weight of mouse.	Remarks.
1908.		
May 9	15.83	Feeding of cakes each containing 0.0015 gm. thyroid begun.
16	14.05	
18	13.94	Acetonitrile, 64.14 mgms., i. e., 4.6 mgms. per gm. mouse. Survived.

B. CONTROLS.

1908.		
May 9	19.00	Feeding of cakes without thyroid begun.
16	19.86	
18	19.52	Acetonitrile, 5.47 mgms., i. e., 0.28 mgm. per gm. mouse. Died 6 hours.

Summary.—The results of these experiments may be summarized as follows:

	Gm. thyroid fed daily.	Mgm. I in thyroid.	Weight of mouse in gms.	Amount of acetonitrile in mgms.		Result.
				Per mouse.	Per gm. mouse.	
A	0.0015	0.0017	13.94	64.14	4.6	Survived.
B	0	0	19.52	5.47	.28	Died.

The mouse which had received the thyroid recovered from more than sixteen times the relative dose fatal to the controls. A total of less than 15 mgms. of thyroid had led to the physiological neutralization of at least 60.24 mgms. acetonitrile.

SERIES III.

A. SHEEP THYROID; 0.19 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1908.		
March 28	15.93	Feeding of cakes each containing 0.001 gm. thyroid begun.
31	14.88	
April 3	15.20	
7	14.33	
8	14.74	Acetonitrile, 67.80 mgms., i. e., 4.6 mgms. per gm. mouse. Survived.
March 28	16.85	Thyroid as above.
31	14.24	
April 3	14.90	
7	14.40	Acetonitrile, 71.12 mgms., i. e., 4.8 mgms. per gm. mouse. Died 1½ hours.

B. CONTROLS.

1908.		
March 28	14.30	Feeding of cakes without thyroid begun.
31	12.88	
April 3	13.15	Acetonitrile, 5.06 mgms., i. e., 0.4 mgm. per gm. mouse. Survived.
7	12.64	
March 28	15.35	Cakes as above.
31	15.06	
April 3	13.17	
7	12.60	
8	12.04	Acetonitrile, 5.06 mgms., i. e., 0.42 mgm. per gm. mouse. Died 1¾ hours.

Summary.—The results of these experiments may be tabulated s follows:

	Gm. thyroid fed daily.	Mgm. I in thyroid.	Weight of mouse in gms.	Amount of acetonitrile in mgms.		Result.
				Per mouse.	Per gm. mouse.	
	0.001	0.0019	14.74	67.8	4.6	Survived.
	.001	.0019	14.40	71.12	4.8	Died.
	0	0	12.64	5.06	.4	Survived.
	0	0	12.04	5.06	.42	Died.

The mouse which had received the thyroid recovered from about leven times the dose fatal to the controls. A total of less than 0 mgms. of thyroid had enabled the mouse to neutralize about 1.8 mgms. acetonitrile.

SERIES IV.

A. SHEEP THYROID; 0.32 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1908.		
pril 28	18.10	Feeding of cakes, each containing 0.0005 gm. thyroid, begun.
ay 2	19.22	
7	18.91	
8	18.91	Acetonitrile, 62.40 mgms., i. e., 3.3 mgms. per gm. mouse. Survived.
pril 28	13.48	Thyroid as above.
ay 2	14.58	
7	15.03	Acetonitrile, 52.60 mgms., i. e., 3.5 mgms. per gm. mouse. Died 6½ hours.

B. CONTROLS.

Date.	Weight of mouse.	Remarks.
1908.		
pril 28	18.98	Feeding of cakes without thyroid begun.
ay 2	20.34	
7	19.53	
8		Acetonitrile, 10.94 mgms., i. e., 0.56 mgm. per gm. mouse. Survived.
pril 28	19.04	Cakes as above.
ay 2	20.63	
7	18.83	
8		Acetonitrile, 10.92 mgms., i. e., 0.58 mgm. per gm. mouse. Died 7 hours.

Summary.—The results of these experiments may be tabulated s follows:

	Gm. thyroid fed daily.	Mgm. I in thyroid.	Weight of mouse in gms.	Amount of acetonitrile in mgms.		Result.
				Per mouse.	Per gm. mouse.	
	0.0005	0.0016	18.91	62.4	3.3	Survived.
	.0005	.0016	15.03	52.6	3.5	Died.
	0	0	19.53	10.94	.56	Survived.
	0	0	18.83	10.92	.58	Died.

The mouse which had received the thyroid recovered from a dose of acetonitrile about six times as large as that fatal to the controls. Less than 5 mgms. of thyroid had led to the physiological neutralization of about 51.8 mgms. of acetonitrile.

SERIES V.

A. HOG THYROID; 0.33 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1907.		
January 9	19.73	Feeding of cakes, each containing 0.0001 gm. thyroid, begun.
14	19.50	
17	18.82	
19	18.80	Acetonitrile, 18.8 mgms., i. e., 1 mgm. per gm. mouse. Survived.
January 9	13.83	Thyroid as above.
14	15.45	
17	14.47	
19	13.65	
20	13.65	Acetonitrile, 17.75 mgms., i. e., 1.3 mgms. per gm. mouse. Died 2¼ hours.

B. CONTROLS.

Date.	Weight of mouse.	Remarks.
1907.		
January 9	16.45	Feeding of cakes without thyroid begun.
14	15.52	
17	15.16	
19	15.07	Acetonitrile, 5.73 mgms., i. e., 0.38 mgm. per gm. mouse. Survived.
January 9	15.25	Cakes as above.
14	15.90	
17	14.70	
19	13.82	
20	13.43	Acetonitrile, 5.51 mgms., i. e., 0.41 mgm. per gm. mouse. Died 5 hours.

Summary.—The results of these experiments may be summarized as follows:

	Gm. thyroid fed daily.	Mgm. I in thyroid.	Weight of mouse in gms.	Amount of acetonitrile in mgms.		Result.
				Per mouse.	Per gm. mouse.	
A	0.0001	0.00033	18.80	18.8	1.0	Survived.
	.0001	.00033	13.65	17.75	1.3	Died.
B	0	0	15.07	5.73	.38	Survived.
	0	0	13.43	5.51	.41	Died.

The mouse which had received thyroid recovered from approximately two and one-half times the dose of acetonitrile fatal to the controls. A total of about 1 mgm. thyroid had enabled the mouse to resist 11.5 mgms. acetonitrile.

We have performed no experiments for the special purpose of determining either the maximum amount of nitrile which may be neutralized under the influence of thyroid or the minimum amount of thyroid which produces a physiological effect. It is evident, however, from the above that as little as 0.1 mgm. of dry thyroid fed daily for eleven days produced a marked effect; still smaller amounts would doubtlessly have produced a distinct effect.

As was shown in a previous paper,[a] this reaction of mice is a very delicate test for thyroid. A few milligrams of thyroid mixed with a large amount of blood or of toasted bread, tamarinds, liquorice powder, etc. (as in the case of a number of the secret "antifat" nostrums), may readily be detected. We have found no other substance with an effect upon the resistance of mice to acetonitrile at all comparable to that of thyroid. This physiological test for thyroid is far more delicate than any chemical method for its detection.[b]

In most of our experiments the thyroid was fed for nine or ten days before the injection of the nitrile. This period was selected in part arbitrarily and in part because we were convinced that it was sufficiently long for the maximum effect of the thyroid to have been produced. A very distinct effect was, however, present in a much shorter time; thus mice which had received rather large amounts of an active thyroid preparation for but two days recovered after the injection of twice the dose of nitrile fatal to the controls.

We have few data as to how long the effect of the thyroid continues after the feeding is discontinued. We have observed in some cases, however, that the increased resistance persisted, although it was less, for at least two weeks.

Conditions influencing the resistance of mice to acetonitrile.—Different groups of mice vary in their susceptibility to acetonitrile; ence in carrying out a series of experiments with this substance it s absolutely indispensable to have mice which have been kept under niform conditions. In testing the effect of thyroid it is also necessary o have a number of controls for each series of experiments. With hese precautions we have rarely failed to obtain entirely concordant esults and have as a rule determined the fatal dose of the nitrile to within 0.01 mgm. per gram body weight. The protocols of the experiments described later show what satisfactory results may, with careful work, be obtained.

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[a] R. Hunt, J. Am. M. Ass., 1907, **49**, p. 240.

[b] As was pointed out in the paper referred to above, this method seemed adapted o throw some light on the question whether there is an excessive amount of thyroid ecretion in the blood in cases of Grave's disease. So far we have been able to test he blood in only three such cases. In one of these the blood had a marked effect in ncreasing the resistance of mice to acetonitrile, indicating an excessive amount of hyroid secretion. In another case the results were doubtful; the amount of blood vas insufficient for even two experiments. In the third case the results were negaive. Possibly this test may have some diagnostic value, although on the thyroid heory of Grave's disease it is not necessary to assume that an excess of thyroid secreion is present in the blood at all times. So far as our observations go it is probable hat the best results would be obtained by administering 1 or 2 c. c. of the blood to nice daily for nine or ten days before testing with the nitrile. Several controls mice fed with normal blood) are indispensable.

It has not been possible to determine all the causes of the differences in susceptibility of different groups of mice to acetonitrile. Diet seems, however, to be the most important single factor; mice kept on certain diets for long periods may be many times as resistant as others of the same age and weight kept on a different diet.

Another very important factor in the toxicity is the season. We have records of experiments performed in each month for three and in some cases for four years. Although the diet was approximately the same throughout the year, the mice were invariably more susceptible to the acetonitrile during the summer than during the winter months. The following table shows the average fatal dose (in milligrams per gram) determined during different months:

January	0.56	May	0.30	September	0.25
February	.35	June	.28	October	.39
March	.58	July	.31	November	.45
April	.53	August	.18	December	.55

There were greater variations (in different series of experiments) in the winter than in the summer months, but in only three cases were the figures as low as the average for the summer months. During the winter months the mice were kept in a room heated to 75° F. (23.9° C.); hence it is not probable that the temperature was the only factor.[a]

On the manner in which thyroid influences the resistance of mice to acetonitrile.—We are unable to suggest any entirely satisfactory explanation of how the feeding of thyroid protects mice against acetonitrile. The effect is the reverse of the one which was anticipated from certain theoretical considerations and which led us to perform the experiments. Inasmuch as the view that one of the functions of the thyroid is to neutralize poisons is very widely held and, since the "neutralization" (or rendering harmless) of acetonitrile in the mouse under the influence of thyroid is the only instance[a] in which a poison is apparently neutralized in any animal—all the other "poisons" to which reference is so frequently made are purely hypothetical—a few words may be devoted to this subject. In the first place, there is certainly no reason to suppose that there is any direct chemical neutralization of the acetonitrile by the thyroid—an interpretation which some have placed upon our previously published results. Not only is it impossible to think of any way in which such a neutralization could occur, but the amounts of the nitrile which may be rendered harmless are so out of proportion to the amounts of thyroid fed that any such interaction is highly improbable. It was shown in the experiments quoted above that from 1 to 20 mgm. of thyroid fed during the course of nine or ten days enabled mice to resist the toxic effects of from five to ten times an equal weight of acetonitrile. By far the greater part of the thyroid is doubtlessly made up of physiologically inert material; hence the disproportion between the amount of poison "neutralized" and the "neutralizing" agent is still greater. It is interesting to compare in this respect the effect of a very active substance—iodothyrine—derived from the thyroid.

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[a] D. W. Harrington (Am. J. Physiol., 1898, **1**, p. 385) has recorded some very interesting experiments on the relation of season to the susceptibility of the guinea pig to anesthetics and operative procedures; the resistance was very low from February to May and highest from October to January. Stimulation of the vagus was more effective in stopping the heart during the fall and winter than during the spring months. Prof. C. W. Edmunds, of the University of Michigan, found frogs to be much less resistant to digitalis in April than during the summer months.

The following statement of F. C. Koch (Proc. Am. Pharm. Ass., 1907, **55**, p. 371) is very interesting in this connection: "I have recently had occasion to determine the per cent of iodine in unadulterated desiccated sheep thyroids prepared at different seasons during the last two and one-half years, and found that the desiccations without exception test as much as three times higher during the winter months than during June and July, and that the per cent gradually diminished toward the summer months and then again gradually increased toward the winter."

Although it is very probable that the differences found by Koch depend upon differences in diet, it is remarkable that the time of the minimum amount of iodine in sheep thyroid should coincide with the period of least resistance of mice to acetonitrile; as we shall see later the resistance of animals to acetonitrile is closely dependent upon the amount of iodine which their thyroids contain.

Experiment, February 10, 1908: The iodothyrine used in this experiment was a commercial preparation (Baeyer) stated to contain 1 part of iodothyrine mixed with 309 parts of milk sugar;[b] it contained 0.014 per cent iodine.

A. IODOTHYRINE (BAEYER).

Date.	Weight of mouse.	Remarks.
1908.		
February 1....	19.43	Feeding of cakes each containing 0.005 gm. iodothyrine begun.
4....	18.60	
8....	17.55	
9....	17.30	Acetonitrile, 43.25 mgms., i. e., 2.5 mgms. per gm. mouse. Survived.
February 1....	20.40	Iodothyrine as above.
4....	19.76	
8....	19.70	
10....	19.07	Acetonitrile, 49.58 mgms., i. e., 2.6 mgms. per gm. mouse. Died 1 hour.

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[a] With the exception of two nitriles closely related to acetonitrile (see p. 20–1).

[b] See New and Non-Official Remedies, 3d ed., 1908, p. 74.

B. CONTROLS.

Date.	Weight of mouse.	Remarks.
1908.		
February 1....	18.83	Feeding of cakes without iodothyrine.
4....	20.01	
7....	20.19	
10....	19.02	Acetonitrile, 4.76 mgms., i. e., 0.25 mgm. per gm. mouse. Survived.
February 1....	16.49	Cakes as above.
4....	15.56	
7....	15.79	
10....	16.31	Acetonitrile, 4.24 mgms., i. e., 0.26 mgm. per gm. mouse. Died 10 hours.

Summary.—0.005 gm. of the milk sugar trituration was fed; this mixture contained only 1 part of the true iodothyrine in 310. Hence there was fed daily but 0.0161 mgm. iodothyrine. The results are summarized in the following table:

	Mgm. true iodothyrine fed daily.	Mgm. I in true iodothyrine.	Weight of mouse in gms.	Amount of acetonitrile in mgms.		Result.
				Per mouse.	Per gm. mouse.	
A	0.0161	0.00000225	17.30	43.25	2.5	Recovered.
	.0161	.00000225	19.07	49.58	2.6	Died.
B	0	0	19.02	4.76	.25	Recovered.
	0	0	16.31	4.24	.26	Died.

Thus a mouse (A) which had received iodothyrine recovered from nearly ten times the relative amount of acetonitrile fatal to the controls. Since 0.25 mgm. per gram is the maximum amount of acetonitrile from which a control (B) recovered, we are justified in assuming that this represents the maximum amount of the poison which a mouse of this series could, under normal conditions, neutralize; for a mouse weighing 17.30 gms. (A) this would be a total of 4.33 mgms. The mouse which weighed 17.30 gms. and which had received iodothyrine recovered, however, from 43.25 mgms. Hence we may conclude that the iodothyrine had enabled the mouse to resist in some way the poisonous effects of 38.92 mgms. acetonitrile. The total amount of iodothyrine which the mouse had received in ten days was somewhat less than 0.161 mgm. Thus one part of true iodothyrine fed to a mouse in the course of ten days had enabled it to resist more than 240 times an equal weight of acetonitrile.

Although we speak of the poison being "neutralized" when a mouse, as a result of the administration of thyroid, recovers from several times the fatal dose of acetonitrile, it is very probable that there has been no neutralization whatever. The effect is probably a preventive one—that is, the thyroid in some way prevents the formation of a poison from the nitrile. The basis of this suggestion is the fact that the feeding of thyroid has no effect upon the toxicity of hydrocyanic acid itself, the poison produced from the nitrile.[a] The thyroid seems to alter the metabolism in such a way that the acetonitrile is disposed of without its breaking down into poisonous constituents, as occurs in the normal animal.[b] Were there a direct interaction between the poison and the thyroid the same results would be expected in all classes of animals; but as we shall see, thyroid not only does not protect rats and guinea pigs against acetonitrile, but it increases their susceptibility to this poison.

The activity of the thyroid in relation to acetonitrile is in sharp contrast to that of certain sulphur compounds. The latter are true antidotes for acetonitrile as well as for many other cyanogen compounds.[c] The hydrocyanic acid which is formed from these compounds as well as hydrocyanic acid administered as such is neutralized by sulphur with the formation of sulphocyanate; the latter compound is less toxic to warm-blooded animals than the cyanogen compounds themselves. The action of the sulphur compounds is the same in all classes of animals investigated.

An hypothesis similar to that which was advanced above to explain the manner in which the feeding of thyroid increases the resistance of mice to acetonitrile—that the thyroid simply alters metabolic processes in such a way that a poisonous substance is not produced from the nitrile—seems sufficient to explain what is frequently called the detoxicatory action of the thyroid. In the normal organism the thyroid probably has a directing influence upon metabolism of such a character that all the products of metabolism are converted into useful or at least harmless compounds. In the absence of the thyroid the metabolism probably proceeds along abnormal lines, with the result that substances necessary for the proper functioning of certain organs are not formed; possibly poisonous substances are also produced, although the evidence is against this supposition.[d] This hypothesis seems more reasonable than one assuming that poisonous substances are normally produced and that an organ has been evolved for the purpose of neutralizing them.

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[a] This argument is, of course, by no means conclusive. It is possible that after the feeding of thyroid hydrocyanic acid is produced from the nitrile, but in such a manner or in a place where it can be neutralized by the body, whereas when hydrocyanic acid itself is administered it may not reach the cells or parts of cells where neutralization is possible. A study of the sulphocyanate excretion of thyroid fed mice after the administration of acetonitrile might throw some light on this subject.

[b] Another possibility which may be considered in this connection is that mice may, under the influence of thyroid, utilize acetonitrile for the synthesis of protein or other nitrogen-containing bodies. Latham (Biochem. J., 1908, **3**, p. 193) believes that certain nitriles are utilized in this way in the normal animal.

[c] See R. Hunt, Arch. internat. de pharmacodyn. e. d. ther., 1904, **12**, p. 447.

[d] The blood of thyroidectomized animals is not, according to a number of writers, toxic; *cf.* L. Launoy, Semaine méd., Par., 1908, **28**, p. 382; D. Baldi, Arch. ital. de biol., Turin, 1899, **31**, p. 281.

It seems unnecessary to divide the functions of the thyroid into "metabolic" and "detoxicatory," as is sometimes done. It is sufficient to recognize that the thyroid has certain effects—for the most part unknown—upon metabolism. Under normal conditions these effects lead to the formation of useful substances or promote in other ways cellular activities. When an unusual poison, such as acetonitrile, is introduced into an animal under the influence of thyroid, its fate will depend upon how the animal's metabolism as regards this poison has been affected by the thyroid; the latter alters the metabolism of the mouse in such a way that the acetonitrile is rendered harmless or is prevented from becoming harmful, but it alters the metabolic processes of rats and guinea pigs in such a way that the acetonitrile becomes much more harmful. In a somewhat similar way certain processes of oxidation proceed along definite lines; for instance, when a poison such as ethyl alcohol is introduced into the body these processes of oxidation destroy it, thus not only making it harmless, but the body is enabled to utilize the energy set free. In the case of methyl alcohol, however, although the oxidation may proceed along similar lines, part of the alcohol is converted into substances (formaldehyde and formic acid) more poisonous than the original substance. The fate of acetonitrile in the body of a mouse to which thyroid has been administered may be compared to the fate of ethyl alcohol; that of acetonitrile in the body of a rat which has received thyroid to that of methyl alcohol.

i. EXPERIMENTS WITH "IODINE FREE" THYROID.[a]

The question whether thyroid free of iodine has any physiological activity has, like the entire question of the relation of iodine to the thyroid, been approached from two points of view. Those who have considered it from the standpoint of the activity of a living gland in

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[a] By "iodine free" thyroid we mean thyroid which does not give even a qualitative test for iodine when 1 gm. of sample is examined by the Baumann method. The limit of this method when applied to a complex body like the thyroid, which contains a very large amount of organic matter, is about 0.01 per cent; although somewhat smaller amounts of iodine could be satisfactorily estimated by using more than 1 gm. of material. As is well known, Paul Bourcet (Compt. rend. Acad. d. sc., Par. 1899, **128,** p. 1120) modified the Baumann method so that very much smaller amounts of iodine could be determined when 50 to 1,000 gms. of the material were employed. The modification consists in concentrating the solution of the fused mass obtained by the incineration of the sample and removing the potassium sulphate by repeated precipitation with alcohol. By this method Bourcet was able to estimate as small an amount as 0.1 mgm. of iodine per kilogram of sample. A. R. Dochez (Johns Hopkins Hospital Bull. 1908, **19,** p. 235) using this method, found iodine in crystallized cane sugar, egg yolk, "C. P. glucose," and in a very large number of other ordinary foods. Of the substances examined only C. P. lactose, egg albumen, chocolate, almonds, and pig embryos (thyroids excised) were reported as containing no iodine.

It is very probable that the thyroid which we call "iodine free" would have been found to contain iodine if examined by Bourcet's modification, and it is also possible the body have assumed that such thyroid is active (Neumeister, Miwa and Stoeltzner, Hutchinson). Those, on the other hand, who have studied the question from the pharmacological side, i. e., in the same way as other drugs are studied, have denied that such thyroid has any physiological activity (Roos, Oswald, Von Cyon and Oswald).

One of the present writers[a] stated in a preliminary paper that he had found thyroid free of iodine to have a low degree of physiological activity. The experiments upon which this statement was based will now be described in detail.

The thyroid most frequently used in these experiments was obtained from infants who had died of various diseases. About 80 such glands were dried at 50° to 60° C., powdered, and thoroughly mixed. There were also available small amounts of thyroid from Maltese kids, an Alaskan bear, and an aoudad.[b] None of these preparations, when examined by the usual method for detecting iodine in the thyroid, gave even a qualitative test for this element.[c]

That the thyroids of children usually contain little or no iodine was first pointed out by Baumann.[d] This investigator found that the presence or absence of iodine and the amount of the latter, when present, varied according to the locality from which the glands were obtained. Nine of twelve thyroids of infants under 1½ years of age, from Freiburg, contained no iodine; three contained from 0.007 to 0.015 per cent. Of six infants from Berlin 1½ years or less of age the thyroids of five contained from 0.014 to 0.033 per cent of iodine; those of the sixth contained none. The thyroids of six children from Hamburg had an average iodine content of 0.07 per cent.

Miwa and Stöltzner[e] examined the thyroids of twelve infants from Berlin; in but one was iodine found, and then only to the extent of 0.025 per cent. Weiss[f] examined the thyroids of seven children under 4¼ years; the iodine varied from traces to 0.037 per cent.

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that these traces of iodine are responsible for the physiological effects we attribute to "iodine free" thyroid. On the other hand, if these thyroids were found to contain traces of iodine it does not follow that this iodine was in the form of combination peculiar to the thyroid.

[a] R. Hunt, J. Am. M. Ass., Chicago, 1907, **49,** 1324.

[b] We are much indebted to Dr. J. R. Mohler, Chief of the Division of Pathology, Bureau of Animal Industry, Department of Agriculture, and pathologist to the National Zoological Park, for the thyroids of these and of a number of other rare animals. The children's thyroids were obtained from Baltimore. In this connection it may be mentioned that H. G. Wells (J. Am. M. Ass., 1897, **29,** p. 1011) found the average percentage of iodine in the thyroid of six adults of Baltimore to be 0.236.

[c] Two gms. of the children's thyroids, 1 of the Maltese kids, 1 of the Alaskan bear, and 0.3 of the aoudad were used for the tests.

[d] E. Baumann, Ztschr. f. physiol. Chem., Strassb., 1896, **22,** p. 11.

[e] S. Miwa and W. Stöltzner, Jahrb. f. Kinderh., 1897, **45,** p. 87.

[f] F. Weiss, München. med. Wchnschr., 1897, **44,** p. 6.

Wells[a] analyzed the thyroids of six children from Chicago under 4 years; three had but traces, the other three from 0.011 to 0.092 per cent. Von Rositzky[b] reported analyses of the thyroids of eight children under 10 years of age; the percentage of iodine varied from 0.012 to 0.041. Oswald[c] found in five children from Basel under 7 years from traces to 0.061 per cent iodine. Charrin and Bourcet[d] found in the thyroid of infants under 3 months who were born of healthy mothers from 0.0004 to 0.0054 per cent iodine. In many cases in which the child or mother had suffered from various diseases no iodine was found. Mendel[e] found no iodine in the thyroids of four infants; that of a fifth contained 0.07 mgm. Jolin[f] found in twenty-seven infants in Sweden under 4 years no distinct traces of iodine in seven cases and from traces to 0.086 per cent in the others. Nagel and Roos[g] found no iodine in the thyroids of four new-born puppies.

The following experiments were performed with the iodine free preparations described above. A few experiments with thyroid containing various amounts of iodine are quoted for comparison. In all cases the dried and powdered gland was used.

Series I.

A. THYROID OF CHILDREN; FREE OF IODINE.

Date.	Weight of mouse.	Remarks.
1906.		
February 24	22.06	Feeding of cakes each containing 0.05 gm. thyroid begun.
March 3	22.85	
6	22.15	Acetonitrile, 17.72 mgms., i. e., 0.8 mgm. per gm. mouse. Survived.
7	....	Acetonitrile, 26.58 mgms., i. e., 1.2 mgms. per gm. mouse. Survived.
February 24	17.22	Thyroid as above.
March 3	18.05	
6	17.75	Acetonitrile, 17.75 mgms., i. e., 1 mgm. per gm. mouse. Survived.
7	....	Acetonitrile, 24.85 mgms., i. e., 1.4 mgms. per gm. mouse. Survived.

B. CONTROLS.

Date.	Weight of mouse.	Remarks.
1906.		
February 21	20.75	Cakes without thyroid.
March 3	19.85	
6	18.75	Acetonitrile, 5.63 mgm., i. e., 0.3 mgm. per gm. mouse. Died 2 hours.
February 21	22.71	Cakes without thyroid.
March 3	22.55	
6	20.95	Acetonitrile, 7.33 mgms., i. e., 0.35 mgm. per gm. mouse. Died 1 to 3 hours.
February 21	23.71	Cakes without thyroid.
March 3	25.35	
6	24.45	Acetonitrile, 12.23 mgms., i. e., 0.5 mgm. per gm. mouse. Died 1 hour.

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[a] H. G. Wells, J. Am. M. Ass., Chicago, 1897, **29**, p. 955.

[b] A. v. Rositzky, Wien. klin. Wchnschr., 1897, **37**, p. 823.

[c] A. Oswald, Ztschr. f. physiol. Chem., Strassb., 1897, **23**, p. 291.

[d] A. Charrin and P. Bourcet, Compt. rend. Acad. d. sc., Par. 1900, **130**, p. 945.

[e] L. B. Mendel, Am. J. Physiol., Boston, 1900, **3**, p. 287.

[f] S. Jolin, Festschrift O. Hammarsten, Upsala, 1906.

[g] W. A. Nagel and E. Roos, Arch. f. (Anat. u.) Physiol., Suppl. Bd., 1902, p. 275.

Summary.—The results of the above experiments may be summarized as follows:

Thyroid.	Gm. thyroid fed daily.	Percentage of iodine in thyroid.	Fatal dose of acetonitrile in mgms. per gm.	
			Recovered.	Died.
A. Children's	0.05	0	1.4	....
B. Controls	....	....	....	0.3

Unfortunately the experiments were not sufficiently numerous for the fatal doses to be determined, but it is evident that the children's thyroid had protected the mice against at least five times the dose of acetonitrile fatal to the controls.

SERIES II.

A. CHILDREN'S THYROID; IODINE FREE.

Date.	Weight of mouse.	Remarks.
1906.		
July 28	20.51	Feeding of cakes each containing 0.05 gm. thyroid begun.
30	20.40	
August 3	19.00	
7	16.80	
9	15.69	Acetonitrile, 31.38 mgms., i. e., 2 mgms. per gm. mouse. Survived.
July 28	21.63	Thyroid as above.
30	20.96	
August 3	21.00	
7	19.00	
9	18.50	
10	18.50	Acetonitrile, 38.85 mgms., i. e., 2.1 mgms. per gm. mouse. Died 1½ hours.

B. SHEEP THYROID; 0.176 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1906.		
July 28	23.71	Feeding of cakes each containing 0.05 gm. thyroid begun.
30	22.79	
August 3	20.39	
7	17.91	Acetonitrile, 25.07 mgms., i. e., 1.4 mgms. per gm. mouse. Survived.
July 28	24.52	Thyroid as above.
30	21.14	
August 3	19.00	
7	15.70	
8	15.25	Acetonitrile, 30.5 mgms., i. e., 2 mgms. per gm. mouse. Survived.
July 28	15.81	Thyroid as above.
30	16.53	
August 3	16.56	
7	17.50	
9	14.33	Acetonitrile, 32.96 mgms., i. e., 2.3 mgms. per gm. mouse. Died 1¾ hours.
July 28	14.11	Thyroid as above.
30	14.36	
August 3	14.08	
4	12.78	
9	12.41	Acetonitrile, 32.27 mgms., i. e., 2.6 mgms. per gm. mouse. Died 2 hours.

C. CONTROLS.

Date.	Weight of mouse.	Remarks.
1906.		
July 28	22.42	Cakes without thyroid
30	23.51	
August 3	24.70	
7	22.83	
8	21.91	Acetonitrile, 3.07 mgms., i. e., 0.14 mgm. per gm. mouse. Survived.
July 28	27.68	Cakes without thyroid.
30	28.02	
August 3	28.73	
7	27.04	
9	24.61	Acetonitrile, 4.18 mgms., i. e., 0.17 mgm. per gm. mouse. Survived.
July 28	22.33	Cakes without thyroid.
30	21.62	
August 3	21.11	
7	18.42	
8	17.58	Acetonitrile, 3.52 mgms., i. e., 0.20 mgm. per gm. mouse. Died 1 to 2 hours.
July 28	21.85	Cakes without thyroid.
30	22.30	
August 3	22.06	
7	20.77	Acetonitrile, 5.19 mgms., i. e., 0.25 mgm. per gm. mouse. Died 1¾ hours.

Summary.—The results of the above experiments may be summarized as follows:

Thyroid.	Gm. thyroid fed daily.	Percentage of iodine in thyroid.	Mgm. I in thyroid fed.	Fatal dose of acetonitrile in mgms. per gm.	
				Recovered.	Died.
A. Children's	0.05	0	0	2.0	2.1
B. Sheep	.05	.176	.088	2.0	2.3
C. Controls				.17	.2

In these experiments both the children's and sheep's thyroids protected against ten times the fatal dose of acetonitrile; the iodine free children's thyroids were as effective as the iodine containing sheep's thyroids. But, as appears from the following experiments, too much thyroid had been given to admit of comparison—that is, the maximum effect had been produced in each case.

In the next series smaller amounts of children's thyroid were fed and the results compared with those obtained with guinea pig's thyroid; the latter contained 0.05 per cent iodine.

SERIES III.

A. CHILDREN'S THYROID; IODINE FREE.

Date.	Weight of mouse.	Remarks.
1907.		
April 5	23. 33	Feeding of cakes each containing 0. 02 gm. thyroid begun.
8	22. 66	
11	21. 42	
15	19. 92	Acetonitrile, 9.96 mgms., i. e., 0.5 mgm. per gm. mouse. Survived.
April 5	23. 35	Thyroids as above.
8	23. 31	
11	23. 95	
15	24. 22	
16	23. 82	Acetonitrile, 14.29 mgms., i. e., 0. 6 mgm. per gm. mouse. Died 2 hours.
April 5	26. 50	Thyroid as above.
8	26. 46	
11	25. 35	
14	24. 32	Acetonitrile, 17.02 mgms., i. e., 0. 7 mgm. per gm. mouse. Died about 9 hours.

B. GUINEA PIG THYROID; 0.05 PER CENT IODINE.

1907.		
April 5	....	Feeding of cakes each containing 0. 0035 gm. thyroid commenced.
8	14. 65	
11	16. 02	
15	15. 62	Acetonitrile, 9.37 mgms., i. e., 0. 6 mgm. per gm. mouse. Survived.
April 5	....	Thyroid as above.
8	18. 72	
11	18. 68	
15	17. 50	
16	17. 82	Acetonitrile, 14.26 mgms., i. e., 0. 8 mgm. per gm. mouse. Survived.
April 5	....	Thyroid as above.
8	17. 85	
11	18. 27	
15	18. 60	Acetonitrile, 18. 60 mgms., i. e., 1 mgm. per gm. mouse. Died 3⅓ hours.

C. CONTROLS.

1907.		
April 5	23. 01	Cakes without thyroid.
8	21. 85	
11	22. 42	
15	22. 52	Acetonitrile, 6.76 mgms., i. e., 0. 3 mgm. per gm. mouse. Survived.
April 5	18. 06	Cakes as above.
8	15. 32	
11	15. 32	
15	14. 90	
16	15. 45	Acetonitrile, 5.41 mgms., i. e., 0. 35 mgm. per gm. mouse. Died 2½ hours.
April 5	18. 01	Cakes as above.
8	15. 36	
11	16. 00	
14	15. 40	Acetonitrile, 6.16 mgms., i. e., 0. 4 mgm. per gm. mouse. Died about 11¾ hours.

Summary.—The results of the above experiments may be summarized as follows:

Thyroid.	Gm. thyroid fed daily.	Percentage of iodine in thyroid.	Mgm. I in thyroid fed.	Fatal dose acetonitrile in mgm. per gm. mouse.	
				Recovered.	Died.
A. Children	0.02	0	0	0.5	0.6
B. Guinea pig	.0035	.05	.0017	.8	1.0
C. Controls				.3	.35

Thus the mice which had received the guinea pig thyroid (B) recovered from about one and five-tenths times the dose of acetonitrile fatal to the mice which had received the children's thyroid (A). Five and seven-tenths times as much of the children's thyroid as of the guinea pig's thyroid had been fed; hence we may conclude that the latter was approximately eight and five-tenths times as active as the former.

SERIES IV.

A. THYROID OF MALTESE KID;[a] NO IODINE.

Date.	Weight of mouse.	Remarks.
1907.		
April 27	12.33	Feeding of cakes each containing 0.05 gm. thyroid begun
30	12.67	
May 3	12.65	
4	12.50	Acetonitrile, 8.75 mgms., i. e., 0.7 mgm. per gm. mouse. Survived.
April 27	20.93	Thyroid as above.
30	19.82	
May 3	20.82	
4	21.67	
7	21.73	
10	22.71	
14	22.95	Acetonitrile, 20.66 mgms., i. e., 0.9 mgm. per gm. mouse. Died 2 hours.
April 27	22.53	Thyroid as above.
30	19.51	
May 3	21.87	
4	22.42	
7	22.61	
10	22.91	
13	22.85	Acetonitrile, 27.42 mgms., i. e., 1.2 mgms per gm. mouse. Died 4 hours.

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[a] Unfortunately no microscopic examination was made of these glands. They weighed 17.65 gms. fresh; 3.25 gms. dry. Thus more than 81.5 per cent consisted of water. They were apparently abnormal (glandular hyperplasia?).

B. CHILDREN'S THYROID; IODINE FREE.

Date.	Weight of mouse.	Remarks.
1907.		
April 27	11.90	Feeding of cakes each containing 0.05 gm. thyroid begun.
30	12.55	
May 3	12.76	
4	12.27	Acetonitrile, 8.59 mgms., i. e., 0.7 mgm. per gm. mouse. Survived.
April 27	15.01	Thyroid as above.
30	15.15	
May 3	15.45	
4	15.58	
7	15.67	
10	15.21	
13	14.39	Acetonitrile, 14.39 mgms., i. e., 1 mgm. per gm. mouse. Survived.
April 27	20.11	Thyroid as above.
30	19.27	
May 3	18.60	
4	18.25	
7	18.17	
10	17.71	
14	15.83	Acetonitrile, 18.99 mgms., i. e., 1.2 mgms. per gm. mouse. Survived.

C. GUINEA PIG THYROID; 0.05 PER CENT IODINE.

1907.		
April 27	13.73	Feeding of cakes each containing 0.007 gm. thyroid begun.
30	13.00	
May 3	12.42	
4	12.80	Acetonitrile, 10.24 mgms., i. e., 0.8 mgm. per gm. mouse. Survived.
April 27	12.65	Thyroid as above.
30	13.42	
May 3	13.32	
7	12.76	
10	12.01	
13	11.47	Acetonitrile, 11.47 mgms., i. e., 1 mgm. per gm. mouse. Survived.
April 27	17.12	Thyroid as above.
30	17.40	
May 3	17.12	
4	16.25	
7	15.61	
10	15.83	
13	15.20	Acetonitrile, 22.80 mgms., i. e., 1.5 mgms. per gm. mouse. Survived.
April 27	24.35	Thyroid as above.
30	24.83	
May 3	24.32	
4	24.06	
7	24.56	
10	24.53	
14	24.67	Acetonitrile, 49.34 mgms., i. e., 2 mgms. per gm. mouse. Survived.

D. CONTROLS.

Date.	Weight of mouse.	Remarks.
1907.		
April 27	13.63	Cakes without thyroid.
May 3	13.95	
4	13.52	Acetonitrile, 3.38 mgms., i. e., 0.25 mgm. per gm. mouse. Survived.
April 27	15.62	Cakes as above.
30	16.65	
May 3	15.43	
7	14.91	
10	14.35	
14	14.02	Acetonitrile, 3.93 mgms., i. e., 0.28 mgm. per gm. mouse. Died 2½ hours.
April 27	13.12	Cakes as above.
30	13.07	
May 3	12.33	
4	12.02	
7	11.45	
10	11.55	
13	11.25	Acetonitrile, 3.94 mgms., i. e., 0.35 mgm. per gm. mouse. Died 7 hours.
April 27	20.57	Cakes as above.
30	21.40	
May 3	20.00	
4	20.32	Acetonitrile, 10.16 mgms., i. e., 0.5 mgm. per gm. mouse. Died 10 hours.

Summary.—These results may be tabulated as follows:

Thyroid.	Gm. thyroid fed daily.	Percentage of iodine in thyroid.	Mg. I in thyroid fed.	Fatal dose of acetonitrile in mgms. per gm. mouse.	
				Recovered.	Died.
A. Maltese kid	0.05	0	0	0.7	0.9
B. Children	.05	0	0	1.2	
C. Guinea pig	.007	.05	.0035	2.0	
D. Controls				.25	.28

Unfortunately the experiments were not sufficiently numerous to permit of a determination of the fatal dose in all cases. The guinea pig thyroid (C) protected against more than twice as much acetonitrile as did the thyroid of the Maltese kid (A); since seven times as much of the latter as of the former was given, we may consider that the guinea pig thyroid is more than fourteen times as active as that of the Maltese kid. Another point of interest in this series is that the children's thyroid (B) was more active than the Maltese kid thyroid; this shows that there are differences in activity of different "iodine free" thyroids.

Owing to lack of material but few experiments were made with smaller amounts of children's thyroid and the results were not altogether satisfactory; 1 mgm. fed daily for ten days had slight, if any, effect.

SERIES V.

A. THYROID OF ALASKAN BEAR; IODINE FREE.

Date.	Weight of mouse.	Remarks.
1908.		
May 19	16.54	Feeding of cakes each containing 0.005 gm. thyroid begun.
23	15.49	
27	15.25	Acetonitrile, 6.87 mgms., i. e., 0.45 mgm. per gm. mouse. Survived.
May 19	11.83	Thyroid as above.
23	11.35	
27	10.13	
28		Acetonitrile, 4.86 mgms., i. e., 0.48 mgm. per gm. mouse. Survived.
May 19	16.75	Thyroid as above.
23	16.77	
27	16.23	Acetonitrile, 8.44 mgms., i. e., 0.52 mgm. per gm. mouse. Died 2½ hours.
May 19	17.43	Thyroid as above.
23	16.66	
27	16.33	Acetonitrile, 9.80 mgms., i. e., 0.60 mgm. per gm. mouse. Died 1½ hours.

B. THYROID OF AOUDAD; IODINE FREE.

1908.		
May 19	15.39	Feeding of cakes each containing 0.005 gm. thyroid begun.
23	17.03	
27	16.00	
28		Acetonitrile, 6.40 mgms., i. e., 0.40 mgm. per gm. mouse. Died 1½ hours.
May 19	15.87	Thyroid as above.
23	15.73	
27	14.65	Acetonitrile, 7.62 mgms., i. e., 0.52 mgm. per gm. mouse. Died 2½ hours
May 19	15.07	Thyroid as above.
23	14.30	
27	13.50	Acetonitrile, 8.10 mgms., i. e., 0.60 mgm. per gm. mouse. Died 2 hours.

C. THYROID OF BLACK LEOPARD; 0.01 PER CENT IODINE.

1908.		
May 19	19.12	Feeding of cakes, each containing 0.005 gm. thyroid begun.
23	18.15	
27	18.99	
28		Acetonitrile, 9.50 mgms., i. e., 0.50 mgm. per gm. mouse. Died 1¼ hours.
May 19	18.03	Thyroid as above.
23	17.32	
27	16.65	Acetonitrile, 11.66 mgms., i. e., 0.70 mgm. per gm. mouse. Died 2¼ hours.
May 19	19.04	Thyroid as above.
23	18.78	
27	18.56	Acetonitrile, 16.70 mgms., i. e., 0.90 mgm. per gm. mouse. Died 3 hours.

D. THYRADEN;[a] 0.085 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1908.		
May 19	15.50	Feeding of cakes each containing 0.00125 gm. thyraden begun.
23	14.81	
27	13.12	Acetonitrile, 9.18 mgms., i. e., 0.7 mgm. per gm. mouse. Survived.
May 19	18.46	Thyroid as above.
23	17.30	
28	16.23	Acetonitrile, 12 98 mgms., i. e., 0.8 mgm. per gm. mouse. Survived.
May 19	15.19	Thyroid as above.
23	15.03	
27	14.65	Acetonitrile, 13.19 mgms., i. e., 0.9 mgm. per gm. mouse. Died 1 hour.
May 19	16.69	Thyroid as above.
23	14.53	
27	14.02	Acetonitrile, 14.02 mgms., i. e., 1 mgm. per gm. mouse. Died 1¼ hours.

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[a] This is a commercial lactose trituration of dried thyroid claimed to be standardized to contain 0.07 per cent iodine. Two specimens examined by us were found to contain slightly more iodine than this, viz, 0.085 per cent.

E. CONTROLS.

1908.		
May 19	15.79	Feeding of cakes without thyroid begun.
23	15.65	
27	15.87	Acetonitrile, 3.97 mgms., i. e., 0.25 mgm. per gm. mouse. Survived.
May 19	15.66	Cakes as above.
23	15.24	
27	16.02	
28	....	Acetonitrile, 4.17 mgms., i. e., 0.26 mgm. per gm. mouse. Died 4¾ hours.
May 19	17.55	Cakes as above.
23	18.15	
26	18.10	Acetonitrile, 5.43 mgms., i. e., 0.30 mgm. per gm. mouse. Died 2 hours.
May 19	11.80	Cakes as above.
23	11.70	
27	10.34	Acetonitrile, 3.31 mgms., i. e., 0.32 mgm. per gm. mouse. Died 3 hours.

Summary.—The results of the above experiments may be summarized as follows:

Thyroid.	Gm. thyroid fed daily.	Percentage of iodine in thyroid.	Mgm. I in thyroid fed.	Fatal dose of acetonitrile in mgm. per gm. mouse.	
				Recovered.	Died.
A. Alaskan bear	0.005	0	0	0.48	0.52
B. Aoudad	.005	0	0		.40
C. Black leopard	.005	.01	.0001		.50
D. Thyraden	.00125	.085	.0011	.80	.90
E. Controls				.25	.26

The iodine free thyroid of the Alaskan bear (A) had some physiological activity, but it was much (perhaps about seven times) less than that of Thyraden (D), with 0.085 per cent iodine. The iodine free thyroid of the aoudad (B) was evidently less active than that of the Alaskan bear; in fact, there is nothing in this series to indicate that it had any activity whatever, but perhaps the doses of acetonitrile were too large. A point of interest is the fact that the thyroid of the black leopard (C), which contained 0.01 per cent iodine, was certainly not more active than the "iodine free" thyroid of the Alaskan bear. Of course it is possible that there was a small amount of iodine in the latter which was overlooked in the analysis; unless this is the case (and the probabilities are that it is not) we must conclude that at times iodine free thyroid is as active as thyroid containing a minimum amount of iodine.

SERIES VI.

A. THYROID OF MALTESE KID; IODINE FREE.

Date.	Weight of mouse.	Remarks.
1908.		
September 5...	17.60	Feeding of cakes each containing 0.01 gm. thyroid commenced.
9...	17.24	
12...	16.90	
14...	16.02	Acetonitrile, 4 mgms., i. e., 0.25 mgm. per gm. mouse. Survived.
September 5...	18.76	Thyroid as above.
9...	18.02	
12...	17.72	
14...	17.13	Acetonitrile, 4.62 mgms., i. e., 0.27 mgm. per gm. mouse. Died 2 hours.
September 5...	18.75	Thyroid as above.
9...	19.70	
12...	19.54	
13...	....	Acetonitrile, 5.86 mgms., i. e., 0.3 mgm. per gm. mouse. Died 2½ hours.
September 5...	19.40	Thyroid as above.
9...	17.30	
12...	17.04	
13...	....	Acetonitrile, 7.67 mgms., i. e., 0.45 mgm. per gm. mouse. Died 1½ hours.

B. THYROID OF ALASKAN BEAR; IODINE FREE.

1908.		
September 5...	17.40	Feeding of cakes each containing 0.01 gm. thyroid commenced.
9...	17.82	
12...	18.88	
14...	19.02	Acetonitrile, 8.56 mgms., i. e., 0.45 mgm. per gm. mouse. Survived.
September 5...	15.24	Thyroid as above.
9...	14.46	
12...	14.80	
13...	....	Acetonitrile, 7.4 mgms., i. e., 0.5 mgm. per gm. mouse. Survived.
September 5...	20.24	Thyroid as above.
9...	20.08	
12...	19.28	
14...	18.94	Acetonitrile, 10.04 mgms., i. e., 0.53 mgm. per gm. mouse. Died 1½ hours.
September 5...	19.00	Thyroid as above.
9...	18.76	
12...	18.00	
13...	....	Acetonitrile, 10.8 mgms., i. e., 0.6 mgm. per gm. mouse. Died 3 hours.

C. THYROID OF CHILDREN; IODINE FREE.

Date.	Weight of mouse.	Remarks.
1908.		
September 5	19.88	Feeding of cakes each containing 0.01 gm. thyroid commenced.
9	20.92	
12	19.94	
13		Acetonitrile, 8.97 mgms., i. e., 0.45 mgm. per gm. mouse. Survived.
September 5	14.84	Thyroid as above.
9	16.20	
12	16.26	
14	15.95	Acetonitrile, 8.45 mgms., i. e., 0.53 mgm. per gm. mouse. Survived.
September 5	17.06	Thyroid as above.
9	15.80	
12	16.00	
14	15.13	Acetonitrile, 8.62 mgms., i. e., 0.57 mgm. per gm. mouse. Died 1½ hours.
September 5	15.68	Thyroid as above.
9	14.42	
12	14.24	
13		Acetonitrile, 8.54 mgms., i. e., 0.6 mgm. per gm. mouse. Died 8 hours.

D. THYROID OF BLACK LEOPARD; 0.01 PER CENT IODINE.

1908.		
September 5	17.14	Feeding of cakes each containing 0.01 gm. thyroid commenced.
9	18.10	
12	17.68	
14	17.23	Acetonitrile, 4.31 mgms., i. e., 0.25 mgm. per gm. mouse. Survived.
September 5	18.08	Thyroid as above.
9	18.22	
12	18.18	
14	17.85	Acetonitrile, 4.82 mgms., i. e., 0.27 mgm. per gm. mouse. Survived.
September 5	19.00	Thyroid as above.
9	19.52	
12	19.24	
13		Acetonitrile, 5.77 mgms., i. e., 0.3 mgm. per gm. mouse. Died 2½ hours.
September 5	17.08	Thyroid as above.
9	16.92	
12	16.78	
13		Acetonitrile, 7.55 mgms., i. e., 0.45 mgm. per gm. mouse. Died 2 hours.

E. THYROID OF SHEEP; 0.125 PER CENT IODINE.

1908.		
September 5	18.56	Feeding of cakes each containing 0.001 gm. thyroid commenced.
9	19.16	
12	18.54	
13		Acetonitrile, 27.81 mgms., i. e., 1.5 mgms. per gm. mouse. Survived.
September 5	19.52	Thyroid as above.
9	19.98	
12	18.16	
14	19.25	Acetonitrile, 30.8 mgms., i. e., 1.6 mgms. per gm. mouse. Survived.
September 5	21.44	Thyroid as above.
9	19.00	
12	18.52	
13		Acetonitrile, 31.48 mgms., i. e., 1.7 mgms. per gm. mouse. Died 1 hour.
September 5	18.00	Thyroid as above.
9	18.43	
12	18.24	
14	16.98	Acetonitrile, 30.56 mgms., i. e., 1.8 mgms. per gm. mouse. Died 2¾ hours.

F. CONTROLS.

Date.	Weight of mouse.	Remarks.
1908.		
September 5...	18.04	Feeding of cakes without thyroid commenced.
9...	17.50	
12...	17.20	
14...	17.00	Acetonitrile, 1.87 mgms., i. e., 0.11 mgm. per gm. mouse. Survived.
September 5...	19.78	Cakes as above.
9...	20.50	
12...	20.56	
14...	20.29	Acetonitrile, 2.64 mgms., i. e., 0.13 mgm. per gm. mouse. Died 2½ hours.
September 5...	21.14	Cakes as above.
9...	20.52	
12...	20.48	
14...	20.22	Acetonitrile, 3.03 mgms., i. e., 0.15 mgm. per gm. mouse. Died 3 hours.
September 5...	21.32	Cakes as above.
9...	22.16	
12...	22.40	
13...	....	Acetonitrile, 4.03 mgms., i. e., 0.18 mgm. per gm. mouse. Died 5 hours.

SUMMARY.

Thyroid.	Gm. thyroid fed daily.	Percentage of iodine in thyroid.	Mgm. I in thyroid fed daily.	Fatal dose of acetonitrile in mgms. per gm.	
				Recovered.	Died.
A. Maltese kid	0.01	0	0	0.25	0.27
B. Alaskan bear	.01	0	0	.50	.53
C. Children	.01	0	0	.53	.57
D. Black leopard	.01	.01	.001	.27	.30
E. Sheep	.001	.125	.00125	1.60	1.70
F. Controls	....	....	....	.11	.13

The three iodine free thyroids (A, B, and C) protected mice against more than twice the dose of acetonitrile fatal to the controls; those of the Alaskan bear and of children were about twice as active as that of the Maltese kid. The thyroid of the black leopard, although it contained a small percentage of iodine, was only about half as active as the thyroids of the Alaskan bear and of the children. One one-thousandth of a gram of sheep thyroid, containing 0.125 per cent iodine, protected against about three times as much acetonitrile as did ten times as much of the iodine free children's thyroid; we may therefore conclude that it was about thirty times as active as the latter.

Series VII.

A. THYROID OF ALASKAN BEAR; IODINE FREE.

Date.	Weight of mouse.	Remarks.
1908.		
June 1	17.06	Feeding of cakes each containing 0.01 gm. thyroid begun.
5	15.45	
9	15.00	
10		Acetonitrile, 3.90 mgms., i. e., 0.26 mgm. per gm. mouse. Survived.
June 1	17.50	Thyroid as above.
5	15.28	
9	15.68	
11	16.32	Acetonitrile, 5.06 mgms., i. e., 0.31 mgm. per gm. mouse. Survived.
June 1	13.50	Thyroid as above.
5	14.02	
9	13.50	Acetonitrile, 5.40 mgms., i. e., 0.40 mgm. per gm. mouse. Died 1¼ hours.
June 1	16.74	Thyroid as above.
5	16.95	
9	17.02	Acetonitrile, 9.36 mgms., i. e., 0.55 mgm. per gm. mouse. Died 4 hours.

B. THYROID OF AOUDAD; IODINE FREE.

Date.	Weight of mouse.	Remarks.
1908.		
June 1	18.94	Feeding of cakes each containing 0.01 gm. thyroid begun.
5	18.52	
9	16.52	
10		Acetonitrile, 3.63 mgms., i. e., 0.22 mgm. per gm. mouse. Died 2 hours.
June 1	24.32	Thyroid as above.
5	23.39	
9	23.46	
10		Acetonitrile, 6.57 mgms., i. e., 0.28 mgm. per gm mouse. Died 1½ hours.
June 1	17.17	Thyroid as above.
5	16.86	
9	16.66	Acetonitrile, 8.33 mgms., i. e., 0.50 mgm. per gm. mouse. Died 1¼ hours.

C. THYROID OF BLACK LEOPARD; 0.01 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1908.		
June 1	15.54	Feeding of cakes each containing 0.01 gm. thyroid begun.
5	14.70	
9	15.14	Acetonitrile, 5.30 mgms., i. e., 0.35 mgm. per gm. mouse. Survived.
June 1	16.72	Thyroid as above.
5	15.07	
9	13.18	
10		Acetonitrile, 5.27 mgms., i. e., 0.40 mgm. per gm. mouse. Died 2¾ hours.
June 1	17.44	Thyroid as above.
5	16.07	
9	15.87	
10		Acetonitrile, 7.14 mgms., i. e., 0.45 mgm. per gm mouse. Died 2¾ hours.
June 1	19.12	Thyroid as above.
5	17.72	
9	18.33	Acetonitrile, 10.08 mgms., i. e., 0.55 mgm. per gm. mouse. Died 1¾ hours.

D. THYROID OF CAT; 0.08 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1908.		
June 1	16.56	Feeding of cakes each containing 0.001 gm. thyroid begun.
5	15.05	
9	14.00	Acetonitrile, 7 mgms., i e., 0.5 mgm. per gm. mouse. Survived.
June 1	14.08	Thyroid as above.
5	14.34	
9	14.44	Acetronitrile, 10.11 mgm., i. e., 0.7 mgm. per gm. mouse. Survived.
June 1	19.29	Thyroid as above.
5	18.34	
9	17.86	
10		Acetonitrile, 15.18 mgms., i e., 0.85 mgm. per gm. mouse. Died 1½ hours.
June 1	17.29	Thyroid as above.
5	16.50	
9	15.68	
10		Acetonitrile, 15.68 mgms., i. e., 1 mgm. per gm. mouse. Died 2 hours.

E. THYRADEN; 0.085 PER CENT IODINE.

1908.		
June 1	14.18	Feeding of cakes each containing 0.001 gm. thyraden begun.
5	12.25	
9	11.88	Acetronitrile, 8.39 mgms., i. e., 0.7 mgm. per gm. mouse. Survived.
June 1	17.04	Thyraden as above.
5	16.22	
9	14.78	Acetonitrile, 11.23 mgms., i. e., 0.76 mgm. per gm. mouse. Survived.
June 1	16.02	Thyraden as above.
5	15.56	
9	15.94	
10		Acetonitrile, 13.55 mgms., i. e., 0.85 mgm. per gm. mouse. Died 3¼ hours.
June 1	24.70	Thyraden as above.
5	23.58	
9	22.50	Acetronitrile, 22.50 mgms., i. e., 1 mgm. per gm. mouse. Died 4 hours.

F. THYROID OF DEER; 0.193 PER CENT IODINE.

1908.		
June 1	18.82	Feeding of cakes each containing 0.001 gm. thyroid begun.
5	17.70	
9	16.60	Acetonitrile, 8.30 mgms., i. e., 0.5 mgm. per gm. mouse. Survived.
June 1	16.16	Thyroid as above.
5	14.50	
9	15.30	Acetonitrile, 10.71 mgms., i. e., 0.7 mgm. per gm. mouse. Survived.
June 1	16.34	Thyroid as above.
5	14.60	
9	15.14	
10		Acetonitrile, 13.63 mgms., i. e., 0.9 mgm. per gm. mouse. Survived.
June 1	17.67	Thyroid as above.
5	15.30	
9	15.54	
10		Acetonitrile, 20.20 mgms., i. e., 1.3 mgms., per gm. mouse. Survived.

G. CONTROLS.

Date.	Weight of mouse.	Remarks.
1908.		
June 1	15.38	Feeding of cakes without thyroid begun.
5	15.10	
9	13.92	Acetonitrile, 2.09 mgms., i. e., 0.15 mgm. per gm. mouse. Survived.
June 1	16.14	Cakes as above.
5	15.42	
9	15.30	
11	15.92	Acetonitrile, 2.55 mgms., i. e., 0.16 mgm. per gm. mouse. Survived.
June 1	16.42	Cakes as above.
5	16.86	
9	15.72	
10	....	Acetonitrile, 2.67 mgms., i. e., 0.17 mgm. per gm. mouse. Survived.
June 1	23.34	Cakes as above.
5	21.88	
9	21.45	
10	....	Acetonitrile, 3.86 mgms., i. e., 0.18 mgm. per gm. mouse. Died 1 hour.
June 1	17.02	Cakes as above.
5	16.00	
9	14.78	
10	....	Acetonitrile, 2.81 mgms., i. e., 0.19 mgm. per gm. mouse. Died 4½ hours.
June 1	18.30	Cakes as above.
5	18.42	
9	17.66	Acetonitrile, 3.53 mgms., i. e., 0.20 mgm. per gm. mouse. Died 1¾ hours.

Summary.—The results of the above experiments may be tabulated as follows:

Thyroid.	Gm. thyroid fed daily.	Percentage of iodine in thyroid.	Mgm. I in thyroid fed daily.	Fatal dose of acetonitrile in mgm. per gm. mouse.	
				Recovered.	Died.
A. Alaskan bear	0.01	0	0	0.31	0.40
B. Aoudad	.01	0	0	....	.22
C. Black leopard	.01	.01	.001	.35	.40
D. Cat	.001	.08	.0008	.70	.85
E. Thyraden	.001	.085	.00085	.76	.85
F. Deer	.001	.193	.00193	1.30	....
G. Controls	....	....	....	.17	.18

The thyroid of the Alaskan bear (A) had a low degree of physiological activity. Inasmuch as 0.01 gm. (per cake) protected against only about one-half as much acetonitrile as did 0.001 of thyraden (0.085 per cent iodine), we may estimate the physiological activity of the latter to be approximately twenty times as great at that of the former. If the aoudad thyroid (B) had any physiological activity, it was too slight to be demonstrated by these experiments; in any case, it was distinctly weaker than the Alaskan bear thyroid (A). The thyroid of the black leopard (C) with 0.01 per cent iodine was apparently no more active than the iodine free thyroid of the Alaskan bear. The experiments with the cat (D) and deer (F) thyroids show that there is a direct parallelism between the iodine content and the physiological activity of thyroid.

SUMMARY AND GENERAL CONCLUSIONS.

The above experiments show that iodine free thyroid, or what is usually called "iodine free" thyroid, has, when tested by our method, a low degree of physiological activity; this result is contrary to that reported by others. The experiments also show that the activity of such thyroid is much less than that of thyroid containing more than distinct traces (0.01 per cent, for example) of iodine. It is also evident that there are differences in the activity of different "iodine free" thyroids; those of the Alaskan bear and of children were more active than those of the Maltese kid or of the aoudad. This may be explained on either of two suppositions: it may be assumed that the activity of the so-called "iodine free" thyroid is in reality due to iodine which is present in too small amounts to be detected when only small samples are available for analysis and that there is more iodine in the thyroids of the children and of the Alaskan bear than in those of the Maltese kid and of the aoudad. Or we may suppose that the iodine free thyreoglobulin has of itself some activity and that the greater activity of some of the "iodine free" thyroids is due to their containing a larger amount of this thyreoglobublin than do other thyroids. This suggestion would also explain why certain "iodine free" thyroids (children and Alaskan bear) are more active than thyroid (black leopard) containing a minimum amount of iodine. The effect of a large amount of iodine free thyreoglobulin may be greater than that of a small amount of iodine containing thyreoglobulin. In connection with this suggestion attention may be called to the work of Oswald,[a] in which it is shown that the thyreoglobulin varies greatly in amount in different thyroids.

Evidence will be presented in later parts of this bulletin that the physiological activity of the thyroid increases in direct proportion to the amount of iodine contained in proper combination. Some of the bearings (upon the physiology and pathology of the thyroid) of this view that iodine free thyroid has a certain degree of activity and that this is much increased by the presence, in proper combination, of iodine have been discussed by one of the present writers n a previous paper.[b] Another application of this conception may be referred to, although it is not our purpose to discuss fully in this connection these collateral subjects. It was stated in the historical review (p. 13) that one of the arguments advanced against the view that iodine (or rather an iodine containing substance) is an important constituent of the thyroid was the fact that the removal of thyroids which contain no iodine is followed by as severe symptoms as the removal of those which do contain iodine. Such a result would be inexplicable on the view that an iodine compound is an essential constituent of the thyroid; it would, however, be intelligible on the hypothesis that thyroid free of iodine alone has a certain degree of physiological activity. We may suppose that this iodine free or iodine poor thyreoglobulin is sufficient, as a rule, for the ordinary purposes of the organism. Much of the iodine usually found in the thyroid may be considered as one of those "factors of safety" discussed by Meltzer.[a] That the iodine free or iodine poor thyreoglobulin is not, however, always adequate to meet the demands of the body is shown, for example, by the losses suffered by sheep breeders on account of cretin lambs before the extensive use of iodine containing salt.[b]

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[a] A. Oswald, Beitr. z. chem. Physiol. u. Path., Brnschwg., 1902, **2**, p. 545.

[b] R. Hunt, J. Am. M. Ass., Chicago, 1907, **49**, p. 1325.

Another indication that the iodine serves a useful purpose is that there is in general an inverse ratio between the size of the thyroid and the amount of iodine it contains.

ii. COMPARATIVE PHYSIOLOGICAL ACTIVITY OF COMMERCIAL SHEEP THYROIDS.

Inasmuch as most of the commercial thyroid preparations are obtained from the sheep, and as these are official in the United States and several other pharmacopœias, especial attention was given to the comparison of their iodine content and physiological activity. There is a widespread belief among clinicians that commercial thyroid preparations differ considerably in activity; we believe that this opinion is fully justified.

Before describing the results of our experiments a few remarks may be made as to the character of quantitative results which may be expected in a study of the physiological action of drugs. When the great importance, both theoretical and practical, of dosage in medicine is considered, it is remarkable that so little work has been done on the scientific principles underlying it.[c] Not many physicians would assume that by doubling the dose of a drug twice the effect would be produced, yet the relation between successive increments of the dose and the physiological effects is unknown in the case of nearly all drugs. With some drugs, in certain doses, double the amount will produce twice the physiological effect. In other doses an increase of 10 per cent in the amount may produce twice the effect. With still other doses the amount administered may be increased many times before twice the effect is produced.

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[a] S. J. Meltzer, J. Am. M. Ass., Chicago, 1907, **48,** p. 655.

[b] *cf.* D. Marine, Johns Hopkins Hosp. Bull., 1907, **18,** p. 359.

[c] *cf.* Juckuff, Versuche zur Auffindung eines Dosierungsgesetztes, Leipzig, 1895; E. Harnack, München med. Wchnschr., 1896, **43,** p. 1065; J. T. Cash, Brit. Med. J., Lond., 1908, i, p. 1213.

The relation between the percentage of iodine in thyroid and its activity in increasing the resistance of mice to acetonitrile seems to offer an unusually favorable opportunity for a study of this character, but we have not been able to devote special attention to it. A brief discussion of the subject is, however, indispensable for an understanding of our results and the conclusions we draw from them. From the experiments already described (Series II, children's thyroids, p. 35) it is evident that even a very weak thyroid preparation will, when given in a sufficient dose, produce as great a physiological effect as an equal amount of a very active preparation. In other words, there is a limit to the degree of resistance which the body can acquire even under the most favorable circumstances. It is obviously necessary, in comparing the physiological effects of different thyroid preparations, to avoid doses which produce a maximum effect. Also, as the doses approach the maximum, each successive increase produces a relatively smaller effect. A curve expressing these facts graphically is a parabola; at a certain point a preparation with twice the activity of another will produce twice the effect; one with three times the activity would produce three times the effect, etc. The farther removed the doses are from this optimum the less close is the correspondence between the size of the dose of the preparation and its (measurable) physiological effect. The range through which there is a direct relation between the physiological activity of the thyroid and the amount of acetonitrile which may be rendered harmless under its influence seems to be rather great; this doubtlessly accounts for the comparative ease with which we have obtained quantitative results in the following experiments.

In work of this character it is not only permissible but necessary to exclude experiments in which the thyroid produced maximum effects. Thus it would obviously be unwarranted to conclude from the experiments of Series II (p. 35) that the thyroid of children is as active as that of sheep because 0.05 gm. of the former protected against as much acetonitrile as did 0.05 gm. of the latter; in each case the amount fed was probably far above that necessary to produce the maximum effect.

The plan of our experiments was as follows: When the activity of several thyroid preparations was to be compared, they were fed in equal amounts, but care was taken that the quantities administered were such as we believed would produce submaximum effects. It was sometimes necessary to perform two or three series of experiments of this character before the amounts most suitable for comparison could be determined; this was partly due to the fact that different lots of mice reacted differently. Thus with some mice 0.001 gm. each of several thyroid preparations produced a maximum effect; in such a case it was necessary to repeat the experiments using 0.0005 gm. for example. In other cases the physiologica effects produced by the feeding of 0.001 gm. of different thyroi preparations were very slight; in such cases the experiments wer repeated with 0.002 or more gram. When the experiments wer performed in this manner we found that the amount of acetonitril from which the mice recovered under the influence of the thyroi was almost directly proportional to the amount of iodine the thyroid contained; hence we conclude that the physiological activity o thyroid is proportional to the iodine content. The correctness o this conclusion was often confirmed as follows: If it required twic as much acetonitrile to cause the death of mice to which thyroid A had been fed as of those to which an equal amount of thyroid B ha been fed, we found that the fatal dose of the nitrile was about th same in each case when two parts of B on the one hand and on part of A on the other were fed.[a] Results of greater scientifi accuracy could probably have been obtained by a slightly differen method of experimenting. Thus instead of comparing the tota amount of thyroid which produced a given degree of protection, i would probably have been more accurate to first subtract th "maximum ineffective" dose (i. e., the maximum amount of thyroid which could be fed without producing any demonstrable physiological effect)[b] and comparing only the amounts in excess o this dose. Similarly it would have probably been more accurat to subtract from the maximum nonlethal doses of the nitrile th maximum doses from which the mice would have recovered ha they received no thyroid. In this way the amounts of the poiso actually neutralized under the influence of the thyroid would hav been compared. Such refinements of experimentation were, however, not necessary for our purpose, which was simply to determin if there is a parallelism between the iodine content and the physiological activity of the thyroid

It may be well to emphasize again some of the precautions necessary in this work. One of the most important of these is to hav mice which have been kept under uniform conditions; the susceptibility of different lots of mice may vary widely even at the sam season. Hence it is necessary to have a number of controls for eac series of experiments. It is also necessary to work with dose which are not too near either the minimum or the maximum reat care is also required in making the cakes. In some of our xperiments we have worked with 1 part of thyroid mixed with ,000 parts of cracker dust. It is obvious that a uniform distribu- on of such a small amount of thyroid in such a large amount of acker dust by ordinary laboratory methods requires considerable re. That, however, remarkably concordant results may be tained by this method is sufficiently demonstrated by the experi- ents which follow.

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[a] This method of double control is similar to the one adopted by one of us in comparing the physiological activity of suprarenal preparations. (J. Am. M. Ass., 1906, **47**, p. 790.)

[b] *cf.* J. T. Cash, Brit. M. J., Lond., 1908, i, p. 1213.

Series I.

A. SHEEP THYROID; 0.087 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1907.		
ovember 22...	22.63	Feeding of cakes each containing 0.005 gm. thyroid commenced.
26...	22.45	
29...	21.62	
ecember 2...	21.52	Acetonitrile, 38.74 mgms., i. e., 1.8 mgms. per gm. mouse. Survived.
ovember 22...	16.32	Thyroid as above.
26...	16.42	
29...	16.59	
ecember 2...	16.07	
3...	16.32	Acetonitrile, 32.64 mgms., i. e., 2 mgms. per gm. mouse. Died 1½ hours.
ovember 22...	20.39	Thyroid as above.
26...	19.44	
29...	19.10	
ecember 2...	18.59	
3...	18.87	Acetonitrile, 41.5 mgms., i. e., 2.2 mgms. per gm. mouse. Died 2 hours.
ovember 22...	21.79	Thyroid as above.
26...	21.57	
29...	20.70	
ecember 2...	19.63	
3...	18.26	Acetonitrile, 45.65 mgms., i. e., 2.5 mgms. per gm. mouse. Died 1 hour.

B. SHEEP THYROID; 0.17 PER CENT IODINE.

1907.		
ovember 22...	18.52	Feeding of cakes each containing 0.005 gm. thyroid begun.
26...	18.35	
29...	18.33	
ecember 2...	17.04	Acetonitrile, 34.1 mgms., i. e., 2 mgms. per gm. mouse. Survived.
ovember 22...	22.92	Thyroid as above.
26...	21.00	
29...	21.65	
ecember 2...	20.64	Acetonitrile, 57.79 mgms., i. e., 2.8 mgms. per gm. mouse. Survived.
ovember 22...	16.00	Thyroid as above.
26...	16.39	
29...	16.56	
ecember 2...	16.70	
3...	15.74	Acetonitrile, 55.09 mgms., i. e., 3.5 mgms. per gm. mouse. Survived.
ovember 22...	20.28	Thyroid as above.
26...	18.41	
29...	18.35	
ecember 2...	17.48	
3...	17.08	Acetonitrile, 78.3 mgms., i. e., 4 mgms. per gm. mouse. Died ¾ hour.

C. CONTROLS.

Date.	Weight of mouse.	Remarks.
1907.		
November 22...	20.03	Feeding of cakes without thyroid begun.
26...	20.17	
29...	19.85	
December 2...	19.30	Acetonitrile, 7.33 mgms., i. e., 0.38 mgm. per gm. mouse. Survived.
November 22...	21.38	Cakes as above.
26...	20.00	
29...	20.26	
December 2...	19.07	
3...	18.86	Acetonitrile, 8.11 mgms., i. e., 0.43 mgm. per gm. mouse. Survived.
November 22...	22.67	Cakes as above.
26...	20.40	
29...	19.44	
December 2...	19.30	Acetonitrile, 8.69 mgms., i. e., 0.45 mgm. per gm. mouse. Died 4 hours.
November 22...	23.63	Cakes as above.
26...	22.89	
29...	21.65	
December 2...	21.96	
3...	22.32	
4...	22.43	Acetonitrile, 10.54 mgms., i. e., 0.47 mgm. per gm. mouse. Died 2 hours.

Summary.—The results of the above experiments may be summarized as follows:

	Gm. thyroid fed daily.	Percentage of iodine in thyroid.	Mgm. I in thyroid fed daily.	Fatal dose of acetonitrile in mgms. per gm.	
				Recovered.	Died.
A	0.005	0.087	0.00435	1.8	2.0
B	.005	.170	.0085	3.5	4.0
C. Controls				.43	.45

The thyroid with approximately twice the amount of iodine protected against approximately twice the dose of acetonitrile.

SERIES II.

The same thyroid preparations as above were used, but twice as much of the iodine poor as of the iodine rich was given. The experiments were performed upon a different lot of mice.

A. SHEEP THYROID; 0.17 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1907.		
December 5...	16.00	Feeding of cakes each containing 0.002 gm. thyroid begun.
9...	15.67	
12...	16.28	
14...	15.72	
15...	15.80	Acetonitrile, 33.18 mgms., i. e., 2.1 mgms. per gm. mouse. Survived.
December 5...	17.54	Thyroid as above.
9...	18.06	
12...	18.13	
14...	17.91	
15...	17.57	Acetonitrile, 40.41 mgms., i. e., 2.3 mgms. per gm. mouse. Survived.
December 5...	20.08	Thyroid as above.
9...	21.20	
12...	20.92	
14...	20.65	Acetonitrile, 49.56 mgms., i. e., 2.4 mgms. per gm. mouse. Died 2 to 3 hours.
December 5...	15.37	Thyroid as above.
9...	15.16	
12...	15.36	
14...	16.31	Acetonitrile, 48.93 mgms., i. e., 3 mgms. per gm. mouse. Died 5¾ hours.

B. SHEEP THYROID; 0.087 PER CENT IODINE.

1907.		
December 5...	14.00	Feeding of cakes each containing 0.004 gm. thyroid begun.
9...	14.92	
12...	14.42	
14...	14.76	
15...	14.05	Acetonitrile, 28.1 mgms., i. e., 2 mgms. per gm. mouse. Survived.
December 5...	15.17	Thyroid as above.
9...	15.49	
12...	15.37	
14...	15.22	
15...	14.28	Acetonitrile, 32.84 mgms., i. e., 2.3 mgms. per gm. mouse. Survived.
December 5...	16.41	Thyroid as above.
9...	16.59	
12...	16.46	
14...	16.54	
15...	16.30	Acetonitrile, 40.75 mgms., i. e., 2.5 mgms. per gm. mouse. Died 2 hours.
December 5...	19.38	Thyroid as above.
9...	20.86	
12...	21.76	
14...	21.46	Acetonitrile, 60.09 mgms., i. e., 2.8 mgms. per gm. mouse. Died 3½ hours.

C. CONTROLS.[a]

Date.	Weight of mouse.	Remarks.
1907.		
December 5...	17.55	Feeding of cakes without thyroid begun.
9...	17.38	
12...	17.54	
14...	17.93	
15...	17.80	Acetonitrile, 3.03 mgms., i. e., 0.17 mgm. per gm. mouse. Survived.
December 5...	15.33	Cakes as above.
9...	15.88	
12...	16.25	
14...	16.52	
15...	15.97	Acetonitrile, 3.03 mgms., i. e., 0.19 mgm. per gm. mouse. Died 1½ hours.
December 5...	17.31	Cakes as above.
9...	17.91	
12...	17.53	
14...	18.12	Acetonitrile, 3.81 mgms., i. e., 0.21 mgm. per gm. mouse. Died 2½ hours.
December 5...	15.09	Cakes as above.
9...	15.24	
12...	14.85	
14...	15.06	Acetonitrile, 3.77 mgms., i. e., 0.25 mgm. per gm. mouse. Died 6½ hours.

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[a] It will be noticed that the fatal dose of acetonitrile for the mice of this series was less than one-half as large as that for those of the preceding series. Such variations in susceptibility are frequent and are largely the result of diet, differences in age, weight, etc.; it illustrates the necessity for using only those mice which have been kept under identical conditions and also the necessity for many controls.

Summary.—The results of the above experiments may be summarized as follows:

	Gm. thyroid fed daily.	Percentage of iodine in thyroid.	Mgm. I in thyroid fed daily.	Fatal dose of acetonitrile in mgms. per gm.	
				Recovered.	Died.
A	0.004	0.087	0.00348	2.3	2.5
B	.002	.17	.0034	2.3	2.4
C. Controls				.17	.19

When two parts of A (0.087 per cent iodine) and one part of B (0.17 per cent iodine) were fed, equal physiological effects were produced.

SERIES III.

In this series both of the methods referred to above were employed, i. e., equal amounts of thyroid containing different amounts of iodine and different amounts of thyroid containing equal amounts of iodine were given. Unfortunately we are not absolutely certain that the analytical data are entirely correct for one of the preparations, viz, that stated to contain 0.061 per cent of iodine. This was the figure obtained on the analysis of a sample taken from the surface of the bottle; later analyses of the thoroughly mixed contents of the bottle showed 0.1 per cent iodine. Inasmuch as the physiological activity of the surface layer corresponded to that of thyroid containing about 0.061 per cent iodine, and as we have often found that the percentage of iodine in a sample of thyroid can be fairly accurately determined by these physiological tests, we are inclined to believe the analysis correct. Even supposing that the thyroid used contained 0.1 per cent iodine, the results show that there is at least a general parallelism between the iodine content and the physiological activity of the thyroid.

A. SHEEP THYROID; 0.061 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1907.		
November 28...	15.97	Feeding of cakes, each containing 0.002 gm. thyroid, commenced.
30...	15.66	
December 3...	15.45	
6...	15.36	
7...	15.25	Acetonitrile, 13.73 mgms., i. e., 0.9 mgm. per gm. mouse. Survived.
November 28...	18.04	Thyroid as above.
30...	17.82	
December 3...	17.63	
6...	17.19	
8...	17.36	Acetonitrile, 19.1 mgms., i. e., 1.1 mgms. per gm. mouse. Survived.
November 28...	19.99	Thyroid as above.
30...	20.39	
December 3...	21.38	
6...	20.59	
7...	20.64	Acetonitrile, 24.77 mgms., i. e., 1.2 mgms. per gm. mouse. Died, 3 hours.
November 28...	18.89	Thyroid as above.
30...	18.08	
December 3...	17.93	
6...	17.52	
8...	17.88	Acetonitrile, 23.24 mgms., i. e., 1.3 mgms. per gm. mouse. Died, 1 hour.

B. SHEEP THYROID; 0.061 PER CENT IODINE.

1907.		
November 28...	16.32	Feeding of cakes, each containing 0.006 gm. thyroid, commenced.
30...	16.24	
December 3...	15.77	
6...	15.52	
7...	15.72	Acetonitrile, 23.58 mgms., i. e., 1.5 mgms. per gm. mouse. Survived.
November 28...	15.06	Thyroid as above.
30...	13.35	
December 3...	13.50	
6...	13.23	
7...	13.60	Acetonitrile, 32.64 mgms., i. e., 2.4 mgms. per gm. mouse. Survived.
November 28...	17.62	Thyroid as above.
30...	17.46	
December 3...	18.06	
6...	17.78	
8...	19.05	Acetonitrile, 59.06 mgms., i. e., 3.1 mgms. per gm. mouse. Survived.
November 28...	21.43	Thyroid as above.
30...	20.97	
December 3...	20.13	
6...	19.53	
8...	19.32	Acetonitrile, 69.55 mgms., i. e., 3.6 mgms. per gm. mouse. Died, three-fourths hour.

C. SHEEP THYROID; 0.192 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1907.		
November 28	18.07	Feeding of cakes, each containing 0.002 gm. thyroid, commenced.
30	17.85	
December 3	17.50	
6	16.77	
7	17.24	Acetonitrile, 37.93 mgms., i. e., 2.2 mgms. per gm. mouse. Survived.
November 28	16.00	Thyroid as above.
30	16.85	
December 3	16.07	
6	15.47	
7	14.92	Acetonitrile, 41.78 mgms., i. e., 2.8 mgms. per gm. mouse. Survived.
November 28	21.85	Thyroid as above.
30	20.05	
December 3	22.40	
6	20.92	
8	21.16	Acetonitrile, 69.83 mgms., i. e., 3.3 mgms. per gm. mouse. Survived.
November 28	17.67	Thyroid as above.
30	15.59	
December 3	16.07	
6	16.17	
8	15.71	Acetonitrile, 58.13 mgms., i. e., 3.7 mgms. per gm. mouse. Died, one-half hour.

D. CONTROLS.

Date.	Weight of mouse.	Remarks.
1907.		
November 28	21.77	Feeding of cakes without thyroid begun.
30	21.92	
December 3	21.09	
6	20.78	
8	20.57	Acetonitrile, 5.14 mgms., i. e., 0.25 mgm. per gm. mouse. Died, about 2 hours.
November 28	24.50	Cakes as above.
30	23.96	
December 3	24.51	
6	24.32	
8	24.51	Acetonitrile, 7.35 mgms., i. e., 0.3 mgm. per gm. mouse. Died 1½ hours.
November 28	21.86	Cakes as above.
30	21.50	
December 3	21.52	
6	20.74	
8	21.50	Acetonitrile, 7.53 mgms., i. e., 0.35 mgm. per gm. mouse. Died 3 hours.

Summary.—The results of the above experiments may be summarized as follows:

	Gm. thyroid fed daily.	Percentage of iodine in thyroid.	Mgm. I in thyroid fed daily.	Fatal dose of acetonitrile in mgms. per gm.	
				Recovered.	Died.
A	0.002	0.061	0.00122	1.1	1.2
B	.006	.061	.00366	3.1	3.6
C	.002	.192	.00384	3.3	3.7
D. Controls					.25

Two thousandths of a gram of thyroid containing 0.192 per cent iodine protected mice against as much acetonitrile as did 0.006 gm. thyroid containing but 0.061 per cent iodine.

Further, with equal amounts of the two thyroid preparations, that containing 0.192 per cent iodine protected against three times as much acetonitrile as did that containing but 0.061 per cent iodine.

SERIES IV.

A. SHEEP THYROID; 0.1 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1908.		
January 15.....	25.65	Feeding of cakes, each containing 0.001 gm. thyroid, commenced.
18.....	26.80	
21.....	26.52	
24.....	26.50	
26.....	26.50	Acetonitrile, 37.1 mgms., i. e., 1.4 mgms. per gm. mouse. Survived.
January 15.....	21.47	Thyroid as above.
18.....	22.05	
21.....	21.36	
24.....	20.00	Acetonitrile, 30 mgms., i. e., 1.5 mgms. per gm. mouse. Survived.
January 15.....	27.50	Thyroid as above.
18.....	27.88	
21.....	27.92	
24.....	27.88	
25.....	25.50	Acetonitrile, 43.35 mgms., i. e., 1.7 mgms. per gm. mouse. Died, 1½ hours.
January 15.....	22.38	Thyroid as above.
18.....	23.56	
21.....	22.35	
24.....	22.22	
25.....	22.02	Acetonitrile, 44.04 mgms., i e., 2 mgms. per gm. mouse. Died, 2 hours.

B. SHEEP THYROID; 0.19 PER CENT IODINE.

1908.		
January 15.....	27.74	Feeding of cakes each containing 0.001 gm. thyroid commenced.
18.....	27.32	
21.....	27.43	
24.....	26.70	Acetonitrile, 53.4 mgms., i. e., 2 mgms. per gm. mouse. Survived.
January 15.....	20.88	Thyroid as above.
18.....	22.50	
21.....	22.45	
24.....	21.58	
25.....	21.90	Acetonitrile, 54.75 mgms., i. e., 2.5 mgms. per gm. mouse. Survived.
January 15.....	19.30	Thyroid as above.
18.....	19.42	
21.....	18.58	
24.....	18.58	
26.....	19.25	Acetonitrile, 51.98 mgms., i. e., 2.7 mgms. per gm. mouse. Survived.
January 15.....	21.52	Thyroid as above.
18.....	20.91	
21.....	21.22	
24.....	20.69	
26.....	20.93	Acetonitrile, 62.79 mgms., i. e., 3 mgms. per gm. mouse. Died 1 hour.

C. SHEEP THYROID; 0.1 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1908.		
January 15	20.00	Feeding of cakes each containing 0.005 gm. thyroid commenced.
18	20.30	
21	20.27	
24	19.70	Acetonitrile, 68.95 mgms., i. e., 3.5 mgms. per gm. mouse. Survived.
January 15	22.52	Thyroid as above.
18	21.51	
21	21.36	
24	20.72	
25	21.21	Acetonitrile, 95.45 mgms., i. e., 4.5 mgms. per gm. mouse. Survived.
January 15	22.72	Thyroid as above.
18	22.98	
21	22.50	
24	22.22	
26	21.75	Acetonitrile, 108.75 mgms., i. e., 5 mgms. per gm. mouse. Died 1¼ hours.
January 15	21.55	Thyroid as above.
18	22.58	
21	22.68	
24	22.70	
26	22.50	Acetonitrile, 123.75 mgms., i. e., 5.5 mgms. per gm. mouse. Died 2½ hours.

D. SHEEP THYROID; 0.19 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1908.		
January 15	23.13	Feeding of cakes each containing 0.005 gm. thyroid commenced.
18	21.30	
21	22.12	
24	21.06	Acetonitrile, 84.24 mgms., i. e., 4 mgms. per gm. mouse. Survived.
January 15	24.17	Thyroid as above.
18	25.28	
21	24.44	
24	23.40	
26	24.25	Acetonitrile, 133.38 mgms., i. e., 5.5 mgms. per gm. mouse. Died 2 hours.
January 15	21.67	Thyroid as above.
18	21.80	
21	20.90	
24	19.62	
26	19.38	Acetonitrile, 116.3 mgms., i. e., 6 mgms. per gm. mouse. Died 5 hours.

The experiments on the controls for this series were somewhat irregular, but the fatal dose was apparently between 0.4 and 0.5 mg. acetonitrile per gram mouse.

SUMMARY.

	Gm. thyroid fed daily.	Percentage of iodine in thyroid.	Mgm. I in thyroid fed daily.	Fatal dose of acetonitrile in mgms. per gm.	
				Recovered.	Died.
A	0.001	0.1	0.001	1.4	1.7
B	.001	.19	.0019	2 7	3.0
C	.005	.1	.005	4.5	5.0
D	.005	.19	.0095	4.0	5.5
Controls				.4(?)	.5(?)

From A and B it is seen that the thyroid containing 0.19 per cent iodine was nearly twice as active as that containing 0.1 per cent. In C and D the effects (with 0.005 gm. thyroid in each cake) were apparently maximum in each case, so that no difference in the activity of the two preparations was apparent.

SERIES V.

A series very similar to the above is the following:

A. SHEEP THYROID; 0.1 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1908.		
February 11....	22.99	Feeding of cakes each containing 0.001 gm. thyroid commenced.
14....	21.66	
17....	22.07	
20....	21.61	
21....	21.74	Acetonitrile, 34.78 mgms., i. e., 1.6 mgms. per gm. mouse. Survived.
February 11....	23.06	Thyroid as above.
14....	21.13	
17....	21.76	
20....	20.94	
21....	21.77	Acetonitrile, 45.72 mgms., i. e., 2.1 mgms. per gm. mouse. Died 2¼ hours.
February 11....	18.50	Thyroid as above.
14....	17.79	
17....	17.47	
20....	17.43	Acetonitrile, 43.58 mgms., i. e., 2.5 mgms. per gm. mouse. Died 3½ hours.
February 11....	20.14	Thyroid as above.
14....	17.48	
17....	17.81	
20....	17.45	Acetonitrile, 52.35 mgms., i. e., 3 mgms. per gm. mouse. Died 2½ hours.

B. SHEEP THYROIDS; 0.19 PER CENT IODINE.

1908.		
February 11....	16.80	Feeding of cakes each containing 0.001 gm. thyroid commenced.
14....	16.02	
17....	16.41	
20....	17.10	Acetonitrile, 34.2 mgms., i. e., 2 mgms. per gm. mouse. Survived.
February 11....	21.25	Thyroid as above.
14....	20.90	
17....	22.48	
20....	17.13	Acetonitrile, 42.83 mgms., i. e , 2.5 mgms. per gm. mouse. Survived.
February 11....	16.38	Thyroid as above
14....	15.29	
17....	15.09	
20....	15.17	
21....	15.59	•Acetonitrile, 46.77 mgms., i. e., 3 mgms. per gm. mouse. Survived.
February 11....	21.69	Thyroid as above.
14....	20.46	
17....	21.02	
20....	20.64	
21....	20.90	Acetonitrile, 73.15 mgms., i. e., 3.5 mgms. per gm. mouse. Died 1¼ hours.

C. CONTROLS.

Date.	Weight of mouse.	Remarks.
1908.		
February 11....	13. 98	Feeding of cakes without thyroid commenced.
14....	13. 91	
17....	14. 26	
20....	13. 75	
21....	14. 33	Acetonitrile, 4.59 mgms., i. e., 0.32 mgm. per gm. mouse. Survived.
February 11....	19. 48	Cakes as above.
14....	17. 70	
17....	17. 45	
20....	16. 90	
21....	17. 42	Acetonitrile, 6.62 mgms., i. e., 0.38 mgm. per gm. mouse. Survived.
February 11....	14. 98	Cakes as above.
14....	13. 08	
17....	12. 72	
20....	12. 85	
21....	13. 04	Acetonitrile, 7.17 mgms., i. e., 0.55 mgm. per gm. mouse. Died 4½ hours.

Summary.—The results of the above experiments may be summarized as follows:

	Gm. thyroid fed daily.	Percentage of iodine in thyroid.	Mgm. I in thyroid fed daily.	Fatal dose of acetonitrile in mgm. per gm.	
				Recovered.	Died.
A..	0. 001	0. 1	0. 0010	1. 6	2. 1
B..	. 001	. 19	. 0019	3. 0	3. 5
C. Controls..................................	....	....	....	. 38	. 55

SERIES VI.

In this series the effects of a number of commercial thyroid powders with different percentages of iodine were compared; the effects of different amounts were also compared.

A. SHEEP THYROID; 0.11 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1908.		
April 8........	13. 41	Feeding of cakes each containing 0.001 gm. thyroid commenced.
11........	12. 68	
14........	13. 32	
16........	13. 25	Acetonitrile, 26.50 mgms., i. e., 2 mgms. per gm. mouse. Survived
April 8........	12. 82	Thyroid as above.
11........	12. 30	
14........	13. 26	
16........	13. 22	
17........	13. 13	Acetonitrile, 32.83 mgms., i. e., 2.5 mgms. per gm. mouse. Survived.
April 8........	14. 63	Thyroid as above.
11........	14. 72	
14........	14. 06	
16........	13. 54	
17........	13. 22	Acetonitrile, 39.66 mgms., i. e., 3 mgms. per gm. mouse. Died 10 hours.
April 8........	13. 17	Thyroid as above.
11........	13. 50	
14........	12. 90	
17........	13. 02	Acetonitrile, 45.57 mgms., i. e., 3.5 mgms. per gm. mouse. Died 1½ hours.

A¹. SAME PREPARATION AS ABOVE.

Date.	Weight of mouse.	Remarks.
1908.		
April 8	14.30	Feeding of cakes each containing 0.002 gm. thyroid commenced.
11	14.96	
14	14.67	
16	14.20	Acetonitrile, 53.96 mgms., i. e., 3.8 mgms. per gm. mouse. Survived.
April 8	12.31	Thyroid as above.
11	12.44	
14	12.43	
16	12.42	
17	12.39	Acetonitrile, 50.8 mgms., i. e., 4.1 mgms. per gm. mouse. Survived.
April 8	13.21	Thyroid as above.
11	13.12	
14	13.50	
17	13.40	Acetonitrile, 61.64 mgms., i. e., 4.6 mgms. per gm. mouse. Died three-fourths hour.

B. SHEEP THYROID; 0.13 PER CENT IODINE.

1908.		
April 8	18.14	Feeding of cakes each containing 0.001 gm. thyroid commenced.
11	19.19	
14	17.07	
16	17.00	Acetonitrile, 37.4 mgms., i. e., 2.2 mgms. per gm. mouse. Survived.
April 8	17.64	Thyroid as above.
11	19.36	
14	20.48	
16	20.43	
17	20.50	Acetonitrile, 49.2 mgms., i. e., 2.4 mgms. per gm. mouse. Survived.
April 8	16.08	Thyroid as above.
11	17.31	
14	17.15	
16	17.86	Acetonitrile, 48.22 mgms., i. e., 2.7 mgms. per gm. mouse. Died 4 hours.
April 8	12.34	Thyroid as above.
11	13.17	
14	12.86	
17	12.82	Acetonitrile, 35.9 mgms., i. e., 2.8 mgms. per gm. mouse. Survived.

C. SHEEP THYROID; 0.13 PER CENT IODINE.

1908.		
April 8	15.76	Feeding of cakes each containing 0.001 gm. thyroid commenced.
11	15.00	
14	14.97	
16	15.40	Acetonitrile, 33.88 mgms., i. e., 2.2 mgms. per gm. mouse. Survived.
April 8	17.80	Thyroid as above.
11	18.24	
14	18.09	
16	18.16	
17	18.23	Acetonitrile, 47.4 mgms., i. e., 2.6 mgms. per gm. mouse. Survived.
April 8	19.50	Thyroid as above.
11	19.26	
14	19.02	
17	17.28	
18	17.07	Acetonitrile, 49.5 mgms., i. e., 2.9 mgms. per gm. mouse. Survived.
April 8	13.20	Thyroid as above.
11	13.18	
14	12.98	
17	16.86	Acetonitrile, 52.27 mgms., i. e., 3.1 mgms. per gm. mouse. Died 1½ hours.

D. SHEEP THYROID; 0.1 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1908.		
April 8	16.34	Feeding of cakes each containing 0.001 gm. thyroid commenced.
11	16.31	
14	16.06	
16	15.77	Acetonitrile, 36.27 mgms., i. e., 2.3 mgms. per gm. mouse. Survived.
April 8	13.50	Thyroid as above.
11	14.42	
14	14.83	
16	14.43	
17	14.95	Acetonitrile, 40.38 mgms., i. e., 2.8 mgms. per gm. mouse. Survived.
April 8	15.07	Thyroid as above.
11	15.76	
14	14.47	
17	14.22	Acetonitrile, 48.35 mgms., i. e., 3.4 mgms. per gm. mouse. Survived.
April 8	14.12	Thyroid as above.
11	13.92	
14	13.63	
17	12.93	
18	13.42	Acetonitrile, 50.99 mgms., i. e., 3.8 mgms. per gm. mouse. Survived.

D¹. SAME PREPARATION AS ABOVE.

Date.	Weight of mouse.	Remarks.
1908.		
April 8	14.88	Feeding cakes each containing 0.002 gm. thyroid commenced.
11	15.12	
14	14.50	
16	14.50	Acetonitrile, 52.2 mgms., i. e., 3.6 mgms. per gm. mouse. Survived.
April 8	10.94	Thyroid as above.
11	11.30	
14	11.48	
17	10.83	Acetonitrile, 41.15 mgms., i. e., 3.8 mgms. per gm. mouse. Died about 30 hou
April 8	14.94	Thyroid as above.
11	14.82	
14	13.39	
17	12.88	
18	12.13	Acetonitrile, 47.3 mgms., i. e., 3.9 mgms. per gm. mouse. Died 5 hours.
April 8	13.05	Thyroid as above.
11	13.98	
14	14.06	
17	13.75	
18	13.66	Acetonitrile, 56 mgms., i. e., 4.1 mgms. per gm. mouse. Died 1¾ hours.

E. SHEEP THYROID; 0.19 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1908.		
April 8	12.40	Feeding cakes each containing 0.001 gm. thyroid commenced.
11	12.70	
14	12.37	
16	12.26	Acetonitrile, 50.27 mgms., i. e., 4.1 mgms. per gm. mouse. Survived.
April 8	13.59	Thyroid as above.
11	13.76	
14	13.45	
16	13.00	
17	12.85	Acetonitrile, 56.54 mgms., i. e., 4.4 mgms. per gm. mouse. Survived.
April 8	17.55	Thyroid as above.
11	18.12	
14	18.02	
16	19.24	Acetonitrile, 88.5 mgms., i. e., 4.6 mgms. per gm. mouse. Died 1½ hours.
April 8	14.74	Thyroid as above.
11	14.48	
14	14.55	
17	14.56	Acetonitrile, 68.43 mgms., i. e., 4.7 mgms. per gm. mouse. Survived.

F. SHEEP THYROID; 0.19 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1908.		
pril 8	12.02	Feeding of cakes each containing 0.001 gm. thyroid commenced.
11	12.00	
14	11.80	
16	11.11	Acetonitrile, 42.22 mgms., i. e., 3.8 mgms. per gm. mouse. Survived.
pril 8	14.37	Thyroid as above.
11	14.08	
14	13.74	
16	12.90	Acetonitrile, 56.76 mgms., i. e., 4.4 mgms. per gm. mouse. Survived.
pril 8	13.08	Thyroid as above.
11	13.14	
14	13.36	
16	13.52	
17	12.94	Acetonitrile, 62.11 mgms., i. e., 4.8 mgms. per gm. mouse. Died 2½ hours

SUMMARY.

	Gm. thyroid fed daily.	Percentage of iodine in thyroid.	Mgm. I in thyroid fed daily.	Fatal dose of acetonitrile in mgms. per gm.	
				Recovered.	Died.
	0.001	0.11	0.0011	2.5	3.0
¹	.002	.11	.0022	4.1	4.6
	.001	.13	.0013	2.8	2.7
	.001	.13	.0013	2.9	3.1
	.001	.10	.0010	3.8	....
¹	.002	.10	.0020	3.8	3.9
	.001	.19	.0019	4.7	4.6
	.001	.19	.0019	4.4	4.8

In the above series the physiological activity of thyroid contain- ng 0.11, 0.13 (two samples), 0.10, and 0.19 per cent (two samples) of odine was compared; the preparations were from different manu- acturers and were received at different times. Three of the samples vith the lower percentages of iodine produced approximately equal ffects. The two samples with the higher percentages also pro- luced equal effects; the latter were nearly twice as great as those roduced by the former. Moreover, one of the weaker preparations. n twice the amount, produced an effect about equal to that of one f the stronger ones. We encountered in this series one of the few xceptions to the general rule that the physiological activity of a hyroid preparation is parallel to the iodine content. The prepara- ion D, containing 0.1 per cent iodine, was as active as E and F, vhich contained 0.19 per cent iodine. Possibly there was some rror in the weighing of the thyroid or the making of the cakes. In n earlier series with the same preparations the ones with the larger ercentages of iodine were about twice as active as the one with the maller percentage of iodine.

SERIES VII.

In this series the comparative physiological activity of two samples of a commercial preparation, Thyraden, was determined; the results with a sample of desiccated sheep thyroid containing very nearly the same percentage of iodine are introduced for the sake of comparison.

A. THYRADEN (A); 0.085 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1908.		
July 3	15.87	Feeding of cakes each containing 0.001 gm. thyraden commenced.
9	14.43	
11	13.57	
12		Acetonitrile, 8.14 mgms., i. e., 0.6 mgm. per gm. mouse. Survived.
July 3	16.05	Thyraden as above.
9	14.10	
11	14.00	
12		Acetonitrile, 9.24 mgms., i. e., 0.66 mgm. per gm. mouse. Survived.
July 3	14.89	Thyraden as above.
9	13.50	
11	13.33	
12		Acetonitrile, 10 mgms., i. e., 0.75 mgm. per gm. mouse. Died 1½ hours.
July 3	13.42	Thyraden as above.
9	10.41	
11	9.85	Acetonitrile, 9.85 mgms., i. e., 1 mgm. per gm. mouse. Died 1¾ hours.

B. THYRADEN (B); 0.085 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1908.		
July 3	15.38	Feeding of cakes each containing 0.001 gm. thyraden commenced.
9	14.28	
11	14.44	
12		Acetonitrile, 6.50 mgms., i. e., 0.45 mgm. per gm. mouse. Survived.
July 3	11.37	Thyraden as above.
9	10.97	
11	10.60	
12		Acetonitrile, 5.30 mgms., i. e., 0.5 mgm. per gm. mouse. Survived.
July 3	15.00	Thyraden as above.
9	13.98	
11	12.21	
12		Acetonitrile, 6.72 mgms., i. e., 0.55 mgm. per gm. mouse. Died 2½ hours.
July 3	15.83	Thyraden as above.
9	15.20	
11	15.65	Acetonitrile, 10.96 mgms., i. e., 0.7 mgm. per gm. mouse. Died 2½ hours.

C. SHEEP THYROID; 0.092 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1908.		
3	15.90	Feeding of cakes each containing 0.001 gm. thyroid begun.
9	14.84	
11	15.05	
12		Acetonitrile, 6.77 mgms., i. e., 0.45 mgm. per gm. mouse. Survived.
3	14.14	Thyroid as above.
9	13.44	
11	13.50	
12		Acetonitrile, 8.1 mgms., i. e., 0.6 mgm. per gm. mouse. Died 1¼ hours.
3	17.79	Thyroid as above.
9	15.75	
11	15.80	
12		Acetonitrile, 11.85 mgms., i. e., 0.75 mgm. per gm. mouse. Died 2¼ hours.
3	12.67	Thyroid as above.
9	11.82	
11	10.90	Acetonitrile, 11.99 mgms., i. e., 1.1 mgms. per gm. mouse. Died 1½ hours.

D. SHEEP THYROID; 0.092 PER CENT IODINE.

1908.		
3	13.73	Feeding of cakes each containing 0.0005 gm. thyroid commenced.
9	12.52	
11	12.49	
13	11.95	Acetonitrile, 3.94 mgms., i. e., 0.33 mgm. per gm. mouse. Survived.
3	16.90	Thyroid as above.
9	15.74	
11	15.44	
12		Acetonitrile, 5.87 mgms., i. e., 0.38 mgm. per gm. mouse. Died 2¼ hours.
3	14.95	Thyroid as above.
9	13.23	
11	12.92	
12		Acetonitrile, 5.81 mgms., i. e., 0.45 mgm. per gm. mouse. Died 2 hours.

E. THYRADEN (A); 0.085 PER CENT IODINE.

1908.		
3	19.41	Feeding of cakes each containing 0.002 gm. thyraden commenced.
9	17.13	
11	16.02	Acetonitrile, 19.22 mgms., i. e., 1.2 mgms. per gm. mouse. Survived.
3	10.77	Thyraden as above.
9	8.52	
11	8.25	
12		Acetonitrile, 10.72 mgms., i. e., 1.3 mgms. per gm. mouse. Died 2 hours.
3	14.15	Thyraden as above.
9	10.66	
11	9.40	
12		Acetonitrile, 15.24 mgms., i. e., 1.62 mgms. per gm. mouse. Died 2½ hours.

F. THYRADEN (B); 0.085 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1908.		
July 3	12.98	Feeding of cakes each containing 0.002 gm. thyraden commenced.
9	12.42	
11	11.78	
12		Acetonitrile, 12.96 mgms., i. e., 1.1 mgms. per gm. mouse. Survived.
July 3	14.07	Thyraden as above.
9	12.72	
11	12.77	
12		Acetonitrile, 16.6 mgms., i. e., 1.3 mgms. per gm. mouse. Survived.
July 3	14.56	Thyraden as above.
9	13.80	
11	13.27	
12		Acetonitrile, 18.57 mgms., i. e., 1.4 mgms. per gm. mouse. Died 2¾ hours.
July 3	16.24	Thyraden as above.
9	13.80	
11	13.60	Acetonitrile, 20.40 mgms., i. e., 1.5 mgms. per gm. mouse. Died 3¾ hours.

G. CONTROLS.

1908.		
July 3	18.24	Feeding of cakes without thyroid commenced.
9	14.45	
10		Acetonitrile, 3.03 mgms., i. e., 0.21 mgm. per gm. mouse. Survived.
July 3	15.08	Cakes as above.
9	13.50	
11	13.50	
13	12.95	Acetonitrile, 3.5 mgms., i. e., 0.27 mgm. per gm. mouse. Survived.
July 3	15.89	Cakes as above.
9	13.40	
11	13.50	Acetonitrile, 4.05 mgms., i. e., 0.3 mgm. per gm. mouse. Survived.
July 3	18.08	Cakes as above.
9	17.07	
11	17.63	
12		Acetonitrile, 5.47 mgms., i. e., 0.31 mgm. per gm. mouse. Died 4½ hours.
July 3	16.04	Cakes as above.
9	14.12	
11	14.86	
12		Acetonitrile, 5.05 mgms., i. e., 0.34 mgm. per gm. mouse. Died 3¼ hours.

SUMMARY.

	Gm. thyroid fed daily.	Percentage of iodine in thyroid.	Mgm. I in thyroid fed daily.	Fatal dose of tonitrile in mg per gm.	
				Recovered.	Die
A	0.001	0.085	0.00085	0.66	0.7
B	.001	.085	.00085	.50	.5
C	.001	.092	.00092	.45	.
D	.0005	.092	.00046	.33	.3
E	.002	.085	.00170	1.2	1.3
F	.002	.085	.00170	1.3	1.
G. Controls				.3	.3

These experiments show that the physiological activity of the two mples of Thyraden was approximately the same, in harmony with eir equal iodine content. Their activity was not far removed from at of a thyroid preparation containing approximately the same rcentage of iodine. Double the amount of Thyraden protected ainst almost twice the dose of acetonitrile.

SERIES VIII.

The physiological activity of two samples of commercial thyroid blets were compared in this series. The tablets were pulverized d amounts containing definite quantities of thyroid were fed.

A. THYROID; 0.38 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1908.		
ptember 24	18.45	Feeding of cakes each containing 0.001 gm. thyroid commenced.
28	17.42	
tober 2	16.95	Acetonitrile, 23.73 mgms., i. e., 1.4 mgms. per gm. mouse. Survived.
tember 24	22.00	Thyroid as above.
28	22.36	
tober 2	20.82	
3		Acetonitrile, 35.39 mgms., i. e., 1.7 mgms. per gm. mouse. Survived.
tember 24	17.28	Thyroid as above.
28	17.02	
tober 2	15.10	
3		Acetonitrile, 27.18 mgms., i. e., 1.8 mgms. per gm. mouse. Survived.
tember 24	14.70	Thyroid as above.
28	14.66	
tober 2	13.20	Acetonitrile, 26.40 mgms., i. e., 2 mgms. per gm. mouse. Died 3 hours.

B. THYROID; 0.38 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1908.		
tember 24	13.00	Feeding of cakes each containing 0.005 gm. thyroid commenced.
28	13.54	
tober 2	11.75	Acetonitrile, 21.15 mgms., i. e., 1.8 mgms. per gm. mouse. Survived.
tember 24	15.18	Thyroid as above.
28	15.20	
tober 2	11.86	Acetonitrile, 32.20 mgms., i. e., 2.8 mgms. per gm. mouse. Survived.
tember 24	16.40	Thyroid as above.
28	15.72	
tober 2	13.58	
3		Acetonitrile, 47.46 mgms., i. e., 3.5 mgms. per gm. mouse. Survived.
tember 24	15.85	Thyroid as above.
28	16.12	
ober 2	14.49	
3		Acetonitrile, 60.86 mgms., i. e., 4.2 mgms. per gm. mouse. Survived.

C. THYROID; 0.065 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1908.		
September 24	13.35	Feeding of cakes each containing 0.001 gm. thyroid commenced
28	13.34	
October 2	12.54	Acetonitrile, 5.64 mgms., i. e., 0.45 mgm. per gm. mouse. Survived.
September 24	12.70	Thyroid as above.
28	12.36	
October 2	11.58	
3	....	Acetonitrile, 5.79 mgms., i. e., 0.50 mgm. per gm. mouse. Survived.
September 24	16.10	Thyroid as above.
28	16.24	
October 2	15.97	
3	....	Acetonitrile, 9.26 mgms., i. e., 0.58 mgm. per gm. mouse. Died 2¾ hours.
September 24	20.88	Thyroid as above.
28	20.62	
October 2	17.73	Acetonitrile, 12.41 mgms., i. e., 0.7 mgm. per gm. mouse. Died 1 hour.

D. THYROID; 0.065 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1908.		
September 24	19.55	Feeding of cakes each containing 0.005 gm. thyroid commenced.
28	19.90	
October 2	19.13	Acetonitrile, 22.96 mgms., i. e., 1.2 mgms. per gm. mouse. Survived.
September 24	13.89	Thyroid as above.
28	13.70	
October 2	13.60	
3	....	Acetonitrile, 21.76 mgms., i. e., 1.6 mgms. per gm. mouse. Survived.
September 24	18.60	Thyroid as above.
28	18.20	
October 2	17.00	
3	....	Acetonitrile, 32.3 mgms., i. e., 1.9 mgms. per gm. mouse. Survived.
September 24	13.15	Thyroid as above.
28	13.06	
October 2	10.55	Acetonitrile, 21.10 mgms., i. e., 2 mgms. per gm. mouse. Died 2½ hours.

SUMMARY.

	Gm. thyroid fed daily.	Percentage of iodine in thyroid.	Mgm. I in thyroid fed.	Fatal dose of ac tonitrile in mgms per gm.	
				Recovered.	Died.
A	0.001	0.38	0.0038	1.8	2.0
B	.005	.38	.019	4.2	......
C	.001	.065	.00065	.5	.58
D	.005	.065	.00325	1.9	2.00
E. Controls	....	....	....	....	.1

Five times as much thyroid was required in D as in A to produc a given physiological effect; this difference corresponds fairly closel with the differences in the percentages of iodine.

Many other experiments with results similar to the above wer performed; a number of them will be reported later in other connections. More than twenty preparations of commercial desiccate thyroids were examined and, with possibly one exception, thei activity was parallel to the iodine content. In the exceptional case the activity seemed to be somewhat less than was anticipated from the percentage of iodine; possibly the thyroid had been overheated in the process of drying or had undergone some decomposition, although there is little evidence that such treatment will materially reduce the activity of thyroid.

Occasionally we encountered a series of experiments in which irregular results were obtained. In such cases the experiments were repeated at least twice and concordant results were obtained. The irregularities had probably resulted from some error in making the cakes or the amount of thyroid fed had been too large or too small to give results which could be compared, or perhaps the mice had not been kept under uniform conditions before the thyroid was fed.

As pointed out elsewhere,[a] we have been able to foretell with considerable accuracy the iodine content of a thyroid preparation by comparing its physiological activity with that of another preparation the iodine content of which was known.

iii. EXPERIMENTS WITH THYROID FROM VARIOUS ANIMALS.

One of the present writers[b] has shown in a previous publication that there is a parallelism between the amount of iodine and the physiological activity of the thyroid of a number of animals (guinea pig, cat, beef, sheep, hog, etc.). Thus a sample of hog thyroid with 0.33 per cent iodine was about six times as active as one of guinea pig thyroid with 0.05 per cent; a sample of sheep thyroid with 0.176 per cent was approximately three times as active as the guinea pig and but one-half as active as the hog thyroid, etc.

We have been able to extend these observations to the thyroids of a large number of wild animals.[c] The results were similar, but the parallelism was not as strict as when thyroids of the same species were compared. The following illustrations may be given: The thyroid of a deer with 0.193 per cent iodine was about three times as active as that of a Barbary sheep with 0.075 per cent; that of the Barbary sheep was more than twice as active as that of a sea lion with 0.022 per cent iodine. The thyroid of a cat with 0.08 per cent iodine was about as active as that of the Barbary sheep with 0.075 per cent, but it was distinctly more active than that of a dog with 0.111 per cent.

If we accept the view that iodine free thyreoglobulin has a low degree of physiological activity, then such slight discrepancies may be explained on the supposition that different thyroids, with equal percentages of iodine, have unequal amounts of this iodine-free material; the latter has a slight but distinct action.

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[a] R. Hunt and A. Seidell, J. Am. M. Ass., Chicago, 1908, **51**, p. 1385.

[b] R. Hunt, J. Am. M. Ass., Chicago, 1907, **49**, p. 1324.

[c] A few of these results were quoted on pp. 45 and 48.

Results similar to the above were obtained with the thyroid both of normal dogs and of dogs which had been fed potassium iodide or iodoform. We also found a parallelism between the iodine content and the physiological activity of a large number of normal and pathological human thyroids. These results will be published in detail in another connection, but the following summaries of a few experiments with human thyroids may be quoted to show how close is the parallelism between iodine content and physiological activity:

SERIES I.—HUMAN THYROID.

No. of specimen.	Gm. thyroid fed daily.	Percentage of iodine.	Mgm. I in thyroid fed.	Fatal dose of acetonitrile in mgm. per gm.	
				Recovered.	Died.
23927	0.001	0.05	0.0005		0.18
23821	.001	.23	.0023	0.7	.9
23807	.001	.26	.0026	.95	1.0
Controls				.13	.14
SERIES II.—HUMAN THYROID.					
23927	0.001	0.05	0.0005	0.08	0.096
23807	.001	.26	.0026	.6	.66
23785	.001	.45	.0045	.9	1.00
SERIES III.—HUMAN THYROID.					
23927	0.001	0.05	0.0005	0.27	0.28
23821	.001	.23	.0023	2.0	
23785	.001	.45	.0045	3.6	
Controls				.21	.21

b. EXPERIMENTS ON RATS.

The feeding of thyroid to rats lowers their resistance to acetonitrile; the result is thus just the opposite to that which occurs in the case of mice. This was, however, the result we had expected in all classes of animals. For it seemed probable, if acetonitrile is poisonous only as a result of the liberation of hydrocyanic acid from it in the body, that whatever stimulates metabolism would also hasten the decomposition of the nitrile, with a corresponding greater production of hydrocyanic acid. We do not know whether this is the correct explanation of the effect of thyroid feeding in the case of the rat or not. Some light upon this question could probably be obtained by a study of the excretion in the urine of sulphocyanates. In one experiment of this character no difference was found between the amounts of sulphocyanic acid in the urine of normal and thyroid fed rats after the administration of acetonitrile.[a]

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[a] It may be mentioned in this connection that negative results were obtained in similar experiments upon dogs.

In the present work we were chiefly interested in determining if there is a relation between the percentage of iodine in different thyroid preparations and their effect upon the resistance of rats to acetonitrile. The following experiments show that there is such a relation and that the degree of activity is in general parallel with the iodine content:

SERIES I.

A. THYRADEN; 0.085 PER CENT IODINE.

Date.	Weight of rat.	Remarks.
1908.		
June 27	32	Feeding of cakes each containing 0.001 gm. thyraden commenced.
July 1	31	
6	30	Acetonitrile, 30 mgms., i. e., 1 mgm. per gm. rat. Survived.
June 27	65	Thyraden as above.
July 1	68	
6	62	Acetonitrile, 86.8 mgms., i. e., 1.4 mgms. per gm. rat. Survived.
June 27	48	Thyraden as above.
July 1	44	
6	45	
7		Acetonitrile, 76.5 mgms., i. e., 1.7 mgms. per gm. rat. Died 5 hours.
June 27	33	Thyraden as above.
July 1	34	
6	32	
7		Acetonitrile, 57.6 mgms., i. e., 1.8 mgms. per gm. rat. Survived.

B. SHEEP THYROID; 0.11 PER CENT IODINE.

1908.		
June 27	29	Feeding of cakes each containing 0.001 gm. thyroid commenced.
July 1	29	
6	28	
7		Acetonitrile, 25.2 mgms., i. e., 0.9 mgm. per gm. rat. Survived.
June 27	54	Thyroid as above.
July 1	53	
6	54	Acetonitrile, 54 mgms., i. e., 1 mgm. per gm. rat. Survived.
June 27	31	Thyroid as above.
July 1	30	
6	29	Acetonitrile, 31.9 mgms., i. e., 1.1 mgms. per gm. rat. Died 3½ hours.
June 27	63	Thyroid as above.
July 1	61	
6	54	Acetonitrile, 70.2 mgms., i. e., 1.3 mgms. per gm. rat. Died about 3 hours.

C. SHEEP THYROID; 0.21 PER CENT IODINE.

1908.		
June 27	43	Feeding of cakes each containing 0.001 gm. thyroid commenced.
July 1	42	
6	42	Acetonitrile, 1.68 mgms., i. e., 0.04 mgm. per gm. rat. Survived.
June 27	29	Thyroid as above.
July 1	29	
6	28	
7		Acetonitrile, 15.40 mgms., i. e., 0.55 mgm. per gm. rat. Survived.
June 27	40	Thyroid as above.
July 1	41	
6	39	Acetonitrile, 27.3 mgms., i. e., 0.7 mgm. per gm. rat. Died 1½–2 hours.
June 27	61	Thyroid as above.
July 1	58	
6	54	Acetonitrile, 54 mgms., i. e., 1 mgm. per gm. rat. Died 1¼ hours.

D. SHEEP THYROID; 0.11 PER CENT IODINE.

Date.	Weight of rat.	Remarks.
1908.		
June 27	33	Feeding of cakes each containing 0.0005 gm. thyroid commenced.
July 1	32	
6	30	Acetonitrile, 45 mgms., i. e., 1.5 mgms. per gm. rat. Survived.
June 27	29	Thyroid as above.
July 1	30	
6	30	
7		Acetonitrile, 48 mgms., i. e., 1.6 mgms. per gm. rat. Survived.
June 27	46	Thyroid as above.
July 1	42	
6	45	
7		Acetonitrile, 81 mgms., i. e., 1.8 mgms. per gm. rat. Died 4¾ hours.
June 27	58	Thyroid as above.
July 1	57	
6	55	Acetonitrile, 104.5 mgms., i. e., 1.9 mgms. per gm. rat. Died 1¼ hours.

E. CONTROLS.

Date.	Weight of rat.	Remarks.
1908.		
June 27	34	Feeding of cakes without thyroid commenced.
July 1	33	
6	34	Acetonitrile, 102 mgms., i. e., 3 mgms. per gm. rat. Survived.
June 27	30	Feeding of cakes as above.
July 1	31	
6	30	
7		Acetonitrile, 99 mgms., i. e., 3.3 mgms. per gm. rat. Survived.
June 27	31	Cakes as above.
July 1	29	
6	29	
7		Acetonitrile, 104.4, mgms., i. e., 3.6 mgms. per gm. rat. Survived.
June 27	52	Cakes as above.
July 1	49	
6	49	
7		Acetonitrile, 196 mgms., i. e., 4 mgms. per gm. rat. Died 4 hours.

Summary.—The above results may be tabulated as follows:

	Gm. thyroid in each cake.	Percentage of iodine in thyroid.	Mgm. I in thyroid in each cake.	Fatal dose of acetonitrile in mgms. per gm.	
				Recovered.	Died.
A	0.001	0.085	0.00085	1.4	1.7
B	.001	.11	.0011	1.0	1.1
C	.001	.21	.0021	.55	.7
D	.0005	.11	.00055	1.6	1.8
E. Controls				3.6	4.0

These results show that the thyroids with the higher percentages of iodine were more active in diminishing the resistance of rats to acetonitrile than was that with the lowest percentage. One-half of a milligram of thyroid containing 0.11 per cent iodine was nearly as active as a milligram of thyroid with 0.085 per cent iodine.

SERIES II.

Various amounts of the thyroid of several different animals with different percentages of iodine were fed in this series. The total amount of iodine fed did not vary greatly and the physiological effects were not very different.

A. DOG THYROID; 0.104 PER CENT IODINE.

Date.	Weight of rat.	Remarks.
1907.		
July 6	124	Feeding of cakes each containing 0. 0035 gm. thyroid commenced.
8	123	
11	120	
15	119	
17	118	Acetonitrile, 94.4 mgms., i. e., 0.8 mgm. per gm. rat. Survived.
July 6	107	Thyroid as above.
8	104	
11	100	
15	98	
17	98	Acetonitrile, 98 mgms., i. e., 1 mgm. per gm. rat. Died 3 hours.
July 6	73	Thyroid as above.
8	70	
11	68	
15	64	
16	63	Acetonitrile, 81.9 mgms., i. e., 1.3 mgms. per gm. rat. Died about 3 hours.
July 6	79	Thyroid as above.
8	77	
11	74	
15	68	
16	70	Acetonitrile, 126 mgms., i. e., 1.8 mgms. per gm. rat. Died 3½ hours.

B. SHEEP THYROID; 0.176 PER CENT IODINE.

1907.		
July 6	122	Feeding of cakes each containing 0.002 gm. thyroid commenced.
8	119	
11	114	
15	105	
16	105	Acetonitrile, 94.5 mgms., i. e., 0.9 mgm. per gm. rat. Survived.
July 6	106	Thyroid as above.
8	106	
11	104	
15	101	
17	102	Acetonitrile, 102 mgms., i. e., 1 mgm. per gm. rat. Survived.
July 6	73	Thyroid as above.
8	69	
11	66	
15	62	
17	64	Acetonitrile, 76.8 mgms., i. e., 1.2 mgms. per gm. rat. Died 2½ hours.
July 6	76	Thyroid as above.
8	71	
11	70	
15	68	
16	68	Acetonitrile, 102 mgms., i. e., 1.5 mgms. per gm. rat. Died 4 hours.

C. HOG THYROID; 0.33 PER CENT IODINE.

Date.	Weight of rat.	Remarks.
1907.		
July 6	142	Feeding of cakes each containing 0.001 gm. thyroid commenced.
8	144	
11	113	
15	106	
17	103	Acetonitrile, 61.8 mgms., i. e., 0.6 mgm. per gm. rat. Survived.
July 6	88	Thyroid as above.
8	84	
11	81	
15	79	
17	80	Acetonitrile, 64 mgms., i. e., 0.8 mgm. per gm. rat. Died 3 hours.
July 6	85	Thyroid as above.
8	82	
11	79	
15	78	
16	77	
17	78	Acetonitrile, 85.8 mgms., i. e., 1.1 mgms. per gm. rat. Died 3 hours.
July 6	88	Thyroid as above.
8	87	
11	85	
15	83	
16	84	Acetonitrile, 109.2 mgms., i. e., 1.3 mgms. per gm. rat. Died about 8 hours

D. CONTROLS.

Date.	Weight of rat.	Remarks.
1907.		
July 6	68	Feeding of cakes without thyroid commenced.
8	70	
11	70	
15	67	
16	67	Acetonitrile, 174 mgms., i. e., 2.6 mgms. per gm. rat. Survived.
July 6	103	Cakes as above
8	100	
11	100	
15	97	
16	96	Acetonitrile, 268.8 mgms., i. e., 2.8 mgms. per gm. rat. Survived.
July 6	109	Cakes as above.
8	112	
11	98	
15	85	
17	85	Acetonitrile, 246.5 mgms., i. e., 2.9 mgms. per gm. rat. Survived.
July 6	88	Cakes as above.
8	87	
11	86	
15	85	Acetonitrile, 255 mgms., i. e., 3 mgms. per gm. rat. Died 5 hours.
July 6	107	Cakes as above.
8	91	
11	92	
15	92	
16	90	
17	90	
18	85	Acetonitrile, 275.9 mgms., i. e., 3. 1 mgms. per gm. rat. Survived.

SUMMARY.

	Gm. thyroid in each cake.	Percentage of iodine in thyroid.	Mgm. I in thyroid of each cake.	Fatal dose of acetonitrile in mgms. per gm.	
				Recovered.	Died.
A	0.0035	0.104	0.0035	0.8	1.0
B	.002	.176	.0035	1.0	1.2
C	.001	.33	.0033	.6	.8
D. Control				3.1	3.0

These results show that in order to cause approximately equal physiological effects (as measured by the diminished resistance of the rats to acetonitrile) it was necessary to feed larger amounts of the iodine poor than of the iodine rich thyroid.

SERIES III.

In this series the physiological activity of dog thyroid, the iodine content of which had been increased by the administration of iodoform, was determined. As the results of these experiments are given in detail in Part II (p. 103–5) of this bulletin, only a summary will be given here.

	Gm. thyroid in each cake.	Percentage of iodine.	Mgm. I in thyroid of each cake.	Fatal dose of acetonitrile in mgms. per gm.	
				Recovered.	Died.
A	0.0005	0.111	0.00055	1.8	2.1
B	.0005	.300	.00150	1.3	1.5
C	.0015	.111	.00166	1.2	1.3
D	.003	.111	.00333	.9	1.0
E	.001	.300	.00300	.5	.6
F. Controls				2.9	3.0

GENERAL CONCLUSIONS.

The above series of experiments show that there is a close parallelism between the iodine content of the thyroid and its physiological activity as measured by its effect in diminishing the resistance of rats to acetonitrile.

2. EXPERIMENTS WITH MORPHINE.

It has been shown above that the feeding of thyroid causes an alteration in the resistance of animals to acetonitrile. The effect differs in different species; there is an increased resistance in the case of mice and diminished resistance in rats. In both cases, however, the activity of the thyroid is parallel with its iodine content.

Experiments will now be described in which the effect of feeding thyroid upon the resistance of animals to another poison, morphine,[a] was determined. Experiments were made upon mice, rats, and guinea pigs. The resistance of all of these animals to morphine was lowered by the feeding of thyroid and there was a general parallelism between the activity of the different thyroid preparations in this respect and their iodine content.

In performing experiments with morphine it is necessary to observe the precautions noted in the experiments with acetonitrile. Different lots of animals react very differently to morphine; thus in our experiments the fatal dose (by subcutaneous injection) for normal animals has ranged from 0.2 to 0.42 mgm. per gram weight for rats,[a] from 0.16 to 0.45 mgm. for mice,[b] and from 0.6 to 0.75 mgm. for guinea pigs.[c] We were able to find definite relations between the resistance of mice to morphine and the diet which they had received; whether in addition to this effect of diet the age of the animals, their weight or the season, temperature, etc., has an influence we have not been able to definitely determine. Animals which have been kept under uniform conditions react very uniformly to the poison, and it is possible to determine the fatal dose with great accuracy.

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[a] Similar results were obtained in a few experiments with acetyl-morphine ("heroin") and with codeine.

These experiments afford another illustration of the folly of determining the "fatal dose" of a poison at the beginning of a long series of experiments, or, as is often done, accepting such a figure as determined by some other investigator and using it as a "control" in a new series of experiments.

a. Experiments on White Rats.

Series I.

A. SHEEP THYROID; 0.176 PER CENT IODINE.

Date.	Weight of rat.	Remarks.
1907.		
May 30	87	Feeding of cakes each containing 0.005 gm. thyroid begun.
June 3	81	
7	77	
9	75	Morphine sulphate, 2.25 mgms., i. e., 0.03 mgm. per gm. rat. Survived.
May 30	88	Thyroid as above.
June 3	84	
7	79	
9	77	Morphine sulphate, 3.85 mgms., i. e., 0.05 mgm. per gm. rat. Survived.
May 30	107	Thyroid as above.
June 3	98	
7	94	
9	92	Morphine sulphate, 6.44 mgms., i. e., 0.07 mgm. per gm. rat. Survived.

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[a] T. Sollmann (Textbook of Pharmacology, 2d ed., 1906, p. 954) places the fatal dose of morphine for rats at 0.45 mgm. per gram body weight.

[b] J. Morgenroth (Berl. klin. Wchnschr., 1903, **40**, p. 471) found the fatal dose of morphine hydrochloride for mice in Frankfort on the Main, to be about 0.93 mgm. per gram.

[c] T. Sollmann (l. c.) places the fatal dose of morphine for guinea pigs at 0.7 mgm. per gram.

B. HOG THYROID; 0.33 PER CENT IODINE.

Date.	Weight of rat.	Remarks.
1907.		
May 30	78	Feeding of cakes each containing 0.005 gm. thyroid begun.
June 3	71	
7	72	
9	67	Morphine sulphate, 1.68 mgms., i. e., 0.025 mgm. per gm. rat. Survived.
May 30	77	Thyroid as above.
June 3	72	
7	70	
9	68	Morphine sulphate, 2.72 mgms., i. e., 0.04 mgm. per gm. rat. Died in 3 hours.
May 30	101	Thyroid as above.
June 3	97	
7	89	
9	90	Morphine sulphate, 4.5 mgms., i. e., 0.05 mgm. per gm. rat. Died $1\frac{3}{4}$ hours.

C: CONTROLS.

Date.	Weight of rat.	Remarks.
1907.		
May 30	93	Feeding of plain cakes begun.
June 3	91	
7	91	
9	91	Morphine sulphate, 27.3 mgms., i. e., 0.3 mgm. per gm. rat. Survived.
May 30	79	Cakes as above.
June 3	75	
7	73	
9	74	Morphine sulphate, 25.9 mgms., i. e., 0.35 mgm. per gm. rat. Survived.
May 30	92	Cakes as above.
June 3	83	
7	82	
9	80	Morphine sulphate, 32.0 mgms., i. e., 0.4 mgm. per gm. rat. Survived.
June 1	46	Cakes as above.
5	45	
7	45	
11	43	Morphine sulphate, 18.06 mgms., i. e., 0.42 mgm. per gm. rat. Died 2 hours.

Summary.—The results of these experiments may be summarized as follows:

	Gm. thyroid in each cake.	Percentage of iodine in thyroid.	Mgm. I in thyroid of each cake.	Average loss of weight in per cent of original weight.	Fatal dose of morphine in mg. per gm.	
					Recovered.	Died.
A	0.005	0.176	0.0088	13.4	0.07	
B	.005	.33	.0165	12.1	.025	0.04
C. Controls.				7.1	.40	.42

Unfortunately the fatal dose of morphine for rats which had received sheep thyroid, A, was not determined; it was probably twice as great as that for those which had received hog thyroids, B. The sheep thyroid contained about one-half as much iodine as the hog thyroid. Rats receiving the hog thyroid died from one-tenth the amount of morphine necessary to kill the controls. The rats of all three series lost weight; the controls lost 7.1 per cent. Evidently the exclusive diet of crackers is not suitable for rats. The loss of weight caused by the thyroid was slight if that which would have probably been caused by the crackers alone is deducted, and probably had nothing to do with the increased susceptibility of the animals to morphine.

SERIES II.

A. THYRADEN; 0.085 PER CENT IODINE.

Date.	Weight of rat.	Remarks.
1908.		
July 14	57	Feeding of cakes each containing 0.001 gm. thyraden begun.
18	56	
22	55	Morphine sulphate, 4.95 mgms., i. e., 0.09 mgm. per gm. rat. Survived.
July 14	54	Thyraden as above.
18	51	
22	49	Morphine sulphate, 5.88 mgms., i. e., 0.12 mgm. per gm. rat. Survived.
July 14	52	Thyraden as above.
18	48	
22	48	Morphine sulphate, 7.20 mgms., i. e., 0.15 mgm. per gm. rat. Died in 5 hours.
July 14	80	Thyraden as above.
18	78	
22	75	
23		Morphine sulphate, 12.0 mgms., i. e., 0.16 mgm. per gm. rat. Survived.

B. SHEEP THYROID; 0.12 PER CENT IODINE.

Date.	Weight of rat.	Remarks.
1908.		
July 14	60	Feeding of cakes each containing 0.001 gm. thyroid begun.
18	55	
22	54	Morphine sulphate, 4.32 mgms., i. e., 0.08 mgm. per gm. rat. Survived.
July 14	66	Thyroid as above.
18	65	
22	66	Morphine sulphate, 7.26 mgms., i. e., 0.11 mgm. per gm. rat. Survived.
July 14	54	Thyroid as above.
18	51	
22	53	
23		Morphine sulphate, 6.89 mgms., i. e., 0.13 mgm. per gm. rat. Survived.
July 14	60	Thyroid as above.
18	58	
22	59	
23		Morphine sulphate, 8.85 mgms., i. e., 0.15 mgm. per gm. rat. Died in 2 hours.

C. SHEEP THYROID; 0.19 PER CENT IODINE.

Date.	Weight of rat.	Remarks.
1908.		
July 14	45	Feeding of cakes each containing 0.001 gm. thyroid begun.
18	41	
22	40	Morphine sulphate, 1.60 mgms., i. e., 0.04 mgm. per gm. rat. Survived.
July 14	42	Thyroid as above.
18	40	
22	38	
23		Morphine sulphate, 1.90 mgms., i. e., 0.05 mgm. per gm. rat. Died 4 hours.
July 14	44	Thyroid as above.
18	43	
22	43	Morphine sulphate, 2.58 mgms., i. e., 0.06 mgm. per gm. rat. Died 4 hours.

D. SHEEP THYROID; 0.2 PER CENT IODINE.

Date.	Weight of rat.	Remarks.
1908.		
July 14	33	Feeding of cakes each containing 0.001 gm. thyroid begun.
18	31	
22	30	Morphine sulphate, 1.50 mgms., i. e., 0.05 mgm. per gm. rat. Survived.
July 14	60	Thyroid as above.
18	54	
22	53	
23	....	Morphine sulphate, 3.18 mgms., i. e., 0.06 mgm. per gm. rat. Died 5 hours.
July 14	65	Thyroid as above.
18	61	
22	62	
23	....	Morphine sulphate, 4.34 mgms., i. e., 0.07 mgm. per gm. rat. Died 2 hours.
July 14	40	Thyroid as above.
18	40	
22	38	Morphine sulphate, 3.04 mgms., i. e., 0.08 mgm. per gm. rat. Died 5½ hours.

E. SHEEP THYROID; 0.19 PER CENT IODINE.

Date.	Weight of rat.	Remarks.
1908.		
July 14	61	Feeding of cakes each containing 0.0005 gm. thyroid begun.
18	59	
22	59	Morphine sulphate, 5.31 mgms., i. e., 0.09 mgm. per gm. rat. Survived.
July 14	87	Thyroid as above.
18	83	
22	81	Morphine sulphate, 8.91 mgms., i. e., 0.11 mgm. per gm. rat. Survived.
July 14	52	Thyroid as above.
18	49	
22	48	Morphine sulphate, 5.76 mgms., i. e., 0.12 mgm. per gm. rat. Died 1¼ hours.
July 14	75	Thyroid as above.
18	71	
22	74	
23	....	Morphine sulphate, 9.62 mgms., i. e., 0.13 mgm. per gm. rat. Died 1½ hours.

F. SHEEP THYROID; 0.2 PER CENT IODINE.

Date.	Weight of rat.	Remarks.
1908.		
July 14	47	Feeding of cakes each containing 0.0005 gm. thyroid begun.
18	46	
22	46	Morphine sulphate, 3.68 mgms., i. e., 0.08 mgm. per gm. rat. Survived.
July 14	48	Thyroid as above.
18	50	
22	47	
23	....	Morphine sulphate, 4.23 mgms., i. e., 0.09 mgm. per gm. rat. Survived.
July 14	50	Thyroid as above.
18	48	
22	49	
23	....	Morphine sulphate, 5.29 mgms., i. e., 0.11 mgm. per gm. rat. Died 3½ hours.
July 14	47	Thyroid as above.
18	45	
22	43	Morphine sulphate, 5.16 mgms., i. e., 0.12 mgm. per gm. rat. Died 12 hours.

All of the controls (i. e., rats which had received no thyroid) died, so that we are unable to state the fatal dose. The smallest dose which killed was 0.2 mgm. per gram.

Summary.—The results of these experiments may be summarized as follows:

	Gm. thyroid in each cake.	Percentage of iodine in thyroid.	Mgm. I in thyroid in each cake.	Loss of weight in per cent of original weight.	Fatal dose of morphine in mgm. per gm.	
					Recovered.	Died.
A	0.001	0.085	0.00085	6.6	0.16	0.15
B	.001	.12	.0012	3.3	.13	.15
C	.001	.19	.0019	7.6	.04	.05
D	.001	.2	.0020	7.6	.05	.06
E	.0005	.19	.00095	4.7	.11	.12
F	.0005	.2	.001	3.6	.09	.11
G controls	...	...	...	1.0	...	.20

The fatal dose of morphine sulphate for the rats which had received the thyroid with the higher percentages of iodine (0.19 to 0.2) was less than one-half as great as that for those which had received the thyroid with the lower percentages of iodine (0.085 and 0.12). The fatal doses were about the same when but one-half as much of the iodine rich thyroid as of the iodine poor thyroid was fed. There were but slight losses of weight.

Series III.

In this series (which was not very complete) the effects of feeding dog thyroid, the iodine content of which had been increased by the administration of iodoform to the dogs, were determined. Since the experiments will be given in detail in Part II (p. 107–9) of this bulletin only the summary is given here.

	Gm. thyroid in each cake.	Percentage of iodine in thyroid.	Mgm. I in thyroid in each cake.	Loss of weight in per cent of original weight.	Fatal dose of morphine in mgm. per gm.	
					Recovered.	Died.
A	0.0005	0.111	0.00055	7.0	0.25	...
B	.0005	.3	.0015	7.6	.20	0.25
C	.0015	.111	.00166	2.4	...	.25
D	.003	.111	.00333	.0	.16	.20
E	.001	.106	.00106	5.5	.25	...
F	.001	.3	.00300	14.0	...	.20
G controls	...	...	...	9.1	.39	.40

These experiments show that with equal amounts of thyroid containing different percentages of iodine those with the higher percentages of iodine caused the greater lowering of resistance to morphine; also that in order to obtain equal physiological effects it was necessary to feed much more of the iodine poor than of the iodine rich thyroid. Loss of weight can not explain the increased susceptibility of the rats to morphine, for with but one exception there was no loss of weight greater than that which could be ascribed to the exclusive cracker diet.

Series IV.

The following experiments may be quoted for the purpose of llustrating how markedly the resistance of rats to morphine may be educed by the feeding of thyroid rather than because they bring out he effects of different iodine percentages. The amounts of thyroid ed were selected so as to make the total amount of iodine approxinately equal in all cases. Unfortunately the experiments were not ufficiently numerous for us to determine the minimum lethal doses n all cases.

A. DOG THYROIDS; 0.111 PER CENT IODINE.

Date.	Weight of rat.	Remarks.
1907.		
une 25	189	Feeding of cakes each containing 0.003 gm. thyroid begun.
29	181	
uly 3	181	
5	182	Morphine sulphate, 7.28 mgms., i. e., 0.04 mgm. per gm. rat. Died 13 hours.
une 25	192	Thyroid as above.
29	186	
uly 3	183	
6	176	Morphine sulphate, 9.68 mgms., i. e., 0.055 mgm. per gm. rat. Died 4 hours.
une 25	152	Thyroid as above.
29	142	
uly 3	138	
5	138	Morphine sulphate, 9.66 mgms., i. e., 0.07 mgm. per gm. rat. Died 9 hours.

B. THYROID OF GUINEA PIG; 0.05 PER CENT IODINE.

1907.		
une 25	116	Feeding of cakes each containing 0.007 gm. thyroid begun.
29	117	
uly 3	118	
5	118	Morphine sulphate, 10.62 mgms., i. e., 0.09 mgm. per gm. rat. Survived.
une 25	156	Thyroid as above.
29	155	
uly 3	155	
5	156	Morphine sulphate, 15.63 mgms., i. e., 0.1 mgm. per gm. rat. Died 3½ hours.
une 25	106	Thyroid as above.
29	101	
uly 3	100	
5	101	Morphine sulphate, 12.12 mgms., i. e., 0.12 mgm. per gm. rat. Died 4½ hours.

C. HOG THYROID; 0.33 PER CENT IODINE.

1907.		
une 25	141	Feeding of cakes each containing 0.001 gm. thyroid begun.
29	134	
uly 3	133	
5	133	Morphine sulphate, 3.99 mgms., i. e., 0.03 mgm. per gm. rat. Died 2¼ hours.
une 25	138	Thyroid as above.
29	134	
uly 3	135	
5	132	Morphine sulphate, 5.28 mgms., i. e., 0.04 mgm. per gm. rat. Died 2¾ hours.
une 25	155	Thyroid as above.
29	149	
uly 3	146	
5	145	Morphine sulphate, 8.7 mgms., i. e., 0.06 mgm. per gm. rat. Died 2 hours.

D. SHEEP THYROID; 0.176 PER CENT IODINE.

Date.	Weight of rat.	Remarks.
1907.		
June 25	114	Feeding of cakes each containing 0.002 gm. thyroid begun.
29	110	
July 3	111	
5	108	Morphine sulphate, 3.24 mgms., i. e., 0.03 mgm. per gm. rat. Died 20 hours.
June 25	158	Thyroid as above.
29	150	
July 3	150	
6	150	Morphine sulphate, 6.75 mgms., i. e., 0.045 mgm. per gm. rat. Died 2 hours.
June 25	127	Thyroid as above.
29	120	
July 3	121	
5	120	Morphine sulphate, 7.2 mgms., i. e., 0.06 mgm. per gm. rat. Died 5½ hours.

E. CONTROLS.

1907.		
June 25	121	Feeding of plain cakes begun.
29	109	
July 3	109	
4	111	Morphine sulphate, 27.75 mgms., i. e., 0.25 mgm. per gm. rat. Survived.
June 25	100	Cakes as above.
29	97	
July 3	96	
4	96	Morphine sulphate, 28.8 mgms., i. e., 0.30 mgm. per gm. rat. Survived.
June 25	119	Cakes as above.
29	120	
July 3	119	
6	119	Morphine sulphate, 36.89 mgms., i. e., 0.31 mgm. per gm. rat. Died 5½ hours.
June 25	73	Cakes as above.
29	71	
July 3	72	
5	70	Morphine sulphate, 22.4 mgms., i. e., 0.32 mgm. per gm. rat. Died 8 hours.
June 25	81	Cakes as above.
29	78	
July 3	74	
5	79	Morphine sulphate, 27.65 mgms., i. e., 0.35 mgm. per gm. rat. Died 2½ hours.

Summary.—The results of the above experiments are shown in the following table:

	Gm. thyroid in each cake.	Percentage of iodine in thyroid.	Mgm. I in thyroid in each cake.	Loss of weight in per cent of original weight.	Fatal dose of morphine in mgm. per gm.	
					Recovered.	Died.
A	0.003	0.111	0.0033	7.0		0.04
B	.007	.05	.0035	.8	0.09	.10
C	.001	.33	.0033	5.5		.03
D	.002	.176	.00352	5.2		.03
E. Controls				4.0	.30	.31

Although the amounts of thyroid fed varied widely (from 0.001 gm. to 0.007 gm.), there was approximately the same amount of iodine in all cases and, with one exception, the lowering of the resistance seemed to be about equal. In all cases except one fully ten times as much morphine was required to kill the controls as to kill those which had received thyroid. The thyroid caused practically no loss of weight; the very slight loss which occurred can be attributed to the cracker diet.

SUMMARY OF EXPERIMENTS ON RATS.

The above experiments show very clearly that the resistance of rats to morphine is lowered by the feeding of thyroid. In two series the fatal dose for the thyroid-fed rats was but one-tenth that for the controls. They also show that the activity of different thyroid preparations in this respect is closely parallel to their iodine content. It would doubtlessly be possible to determine with a fair degree of accuracy the percentage of iodine in a thyroid preparation by comparing its physiological activity with that of another preparation containing a known percentage of iodine. The increased susceptibility to morphine is entirely independent of any loss of weight; it can evidently not be attributed to a "general lowering of resistance" such as may be supposed to occur when there is a loss of weight from starvation.[a]

b.—Experiments on Mice.

The results of the experiments on mice were somewhat irregular. There was invariably, however, an increased susceptibility to morphine as a result of the feeding of thyroid. As a rule the thyroid containing the larger percentage of iodine had the greater effect but there were many exceptions which we are unable to explain. It would probably be impossible to detect small variations in the percentage of iodine in different thyroid preparations from their effects upon the resistance of mice to morphine.

As these experiments do not offer many points of special interest they will be reproduced in abstract only. The average loss of weight, expressed in percentage of original weight, is given in the last column.

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[a] Starvation markedly increases the resistance of animals to some poisons; this is the case, for example, with acetonitrile. (See R. Hunt, Studies in Experimental Alcoholism, Bull. 33, Hygienic Laboratory, 1907, p. 34.)

SERIES I.—SHEEP THYROID.

	Gm. thyroid fed daily.	Percentage of iodine in thyroid.	Mgm. I in thyroid fed daily.	Fatal dose of morphine in mgm. per gm.		Loss in weight in percentage of original weight.
				Recovered.	Died.	
A	0.001	0.085	0.00085	0.17	0.25	9.0
B	.001	.11	.0011	.09	.11	14.3
C	.001	.11	.0011	.12	.16	3.7
D	.001	.19	.0019	.12	.14	10.7
E	.001	.21	.0021	.13	.16	14.6
F. Controls				.19	.20	9.9

The resistance of mice to morphine was uniformly diminished by the thyroid. The thyroid having the least effect was the preparation containing the smallest percentage of iodine; the effects of the other preparations were nearly equal, although some contained nearly twice as much iodine as others. There was no constant relation between the loss of weight and the diminished resistance to morphine.

SERIES II.

	Gm. thyroid fed daily.	Percentage of iodine in thyroid.	Mgm. I in thyroid fed daily.	Fatal dose in mgm. per gm.		Loss in weight.
				Recovered.	Died.	
A	0.005	0.085	0.00425	0.17	0.18	8.6
B	.005	.11	.0055	.08	.11	13.2
C	.005	.19	.0095	.09	.10	8.8
D	.005	.21	.0105	.07	.09	23.5
E	.01	.1	.01	.10	.12	15.1
F	.01	.11	.011	.13	.14	16.5
G	.01	.11	.011	.105	.12	13.6
H	.01	.11	.011	.13	.16	14.2
I	.01	.19	.019	.08	.09	15.0
J	.01	.21	.021	.06	.08	9.4
K. Controls				.17	.18	7.5

The thyroid with the largest percentage of iodine was the most active when the smaller (0.005 gm.) as well as when the larger (0.01 gm.) amounts of thyroid were fed. In some cases the loss of weight was parallel with the increased susceptibility to morphine; in other cases it was not.

SERIES III.

	Gm. thyroid fed daily.	Percentage of iodine in thyroid.	Mgm. I in thyroid fed daily.	Fatal dose in mgm. per gm.		Loss in weight.[a]
				Recovered.	Died.	
A	0.01	0.05	0.005	0.18	0.22	+ 3.0
B	.01	.104	.0104	.11	.16	5.8
C	.01	.19	.0190		.09	18.0
D	.01	.298	.0298	.09	.10	11.7
E	.01	.33	.0330	.07	.09	2.4
F. Controls				.43	.45	+ 3.7

[a] The + sign indicates that the mice had increased in weight.

With the increased percentage of iodine in the thyroid there was, with one exception, a uniform increase in physiological activity, as shown by the increased susceptibility to morphine.

The changes in weight were in most cases not marked; in two instances there was a slight increase. The most marked change was with the mice of group C; the average loss of weight with these was 18 per cent. Perhaps this indicates that the mice of this series had eaten more of the cakes than had the mice of the other series.

The following series may be quoted as additional illustrations of how the susceptibility of mice to morphine may be increased by the administration of thyroid:

SERIES IV.

	Gm. thyroid fed daily.	Percentage of iodine in thyroid.	Mg. I in thyroid fed daily.	Fatal dose in mgm. per gm.		Loss in weight.[a]
				Recovered.	Died.	
A.	0.01	0.19	0.019	0.10	0.15	5.1
B. Controls				.36		+6.8

SERIES V.

	Gm. thyroid fed daily.	Percentage of iodine in thyroid.	Mg. I in thyroid fed daily.	Fatal dose in mgm. per gm.		Loss in weight.[a]
				Recovered.	Died.	
A.	0.03	0.186	0.0558	0.18	0.22	14.2
B. Controls				.38	.43	7.9

SERIES VI.[a]

	Gm. thyroid fed daily.	Percentage of iodine in thyroid.	Mg. I in thyroid fed daily.	Fatal dose in mgm. per gm.		Loss in weight.[a]
				Recovered.	Died.	
A.	0.2	0.186	0.0372	0.07	0.08	1.9
B. Controls				.24	.24	1.5

[a] The + sign indicates that the mice had increased in weight.

[b] The experiment was continued but six days instead of ten, as was usually the case.

SUMMARY OF EXPERIMENTS ON MICE.

The above experiments show that the resistance of mice to morphine was uniformly lowered by the feeding of thyroid. This lowering of the resistance occurred independently of the changes in weight; in some cases there was a markedly increased susceptibility to morphine with an increase in weight. In general the thyroid containing the higher percentages of iodine caused the greatest changes in resistance, but there were a number of exceptions.

c.—Experiments on Guinea Pigs.

Experiments were not made in which the effects of thyroid containing different percentages of iodine upon the resistance of guinea pigs to morphine were determined. The following experiments show that the resistance of these animals to morphine is diminished by the feeding of thyroid. The amount of thyroid was very large; in the second series there was a loss of weight, while in the first there was only a retardation of growth:

SERIES I.

A. SHEEP THYROID; 0.084 PER CENT IODINE.

Date.	Weight of pig.	Remarks.
1907.		
May 1	265	Feeding of thyroid, 0.120 gm. daily, begun.
4	265	
8	260	
10	260	
13	275	Morphine sulphate, 55 mgms., i. e., 0.2 mgm. per gm. pig. Survived.
May 1	330	Thyroid as above.
4	330	
8	320	
11	315	
15	267	Morphine sulphate, 66.75 mgms., i. e., 0.25 mgm. per gm. pig. Died 16 hours.
May 1	285	Thyroid as above.
4	280	
8	280	
11	280	
14	259	Morphine sulphate, 77.7 mgms., i. e., 0.3 mgm. per gm. pig. Died 8½ hours.
May 1	215	Thyroid as above.
4	200	
8	215	
11	215	
14	208	Morphine sulphate, 83.2 mgms., i. e., 0.4 mgm. per gm. pig. Died 3 hours.

B. CONTROLS.

1907.		
May 1	320	
4	335	
9	360	
13	385	
14	348	Morphine sulphate, 174 mgms., i. e., 0.5 mgm. per gm. pig. Survived.
May 1	200	
4	210	
9	220	
13	275	
15	250	Morphine sulphate, 150 mgms., i. e., 0.6 mgm. per gm. pig. Died 3 hours.
May 1	225	
4	240	
9	275	
13	305	Morphine sulphate, 213.5 mgms., i. e., 0.7 mgm. per gm. pig. Died 3¾ hours.

Series II.

A. SHEEP THYROID; 0.084 PER CENT IODINE.

Date.	Weight of pig.	Remarks.
1907.		
April 4	265	Feeding of thyroid, 0.24 gm. daily, begun.
8	232	
12	220	
16	195	Morphine sulphate, 48.75 mgms., i. e., 0.25 mgm. Died 10 to 15 hours.
April 4	244	Thyroid as above.
8	235	
12	255	
14	256	Morphine sulphate, 76.8 mgms., i. e., 0.3 mgm. per gm. pig. Died 21 hours.
April 4	260	Thyroid as above.
8	225	
12	215	
14	217	Morphine sulphate, 108.5 mgms., i. e., 0.5 mgm. per gm. pig. Died 4 hours.

B. CONTROLS.

Date.	Weight of pig.	Remarks.
1907.		
April 4	250	
8	270	
12	300	
14	302	Morphine sulphate, 196.3 mgms., i. e., 0.65 mgm. per gm. pig. Survived.
April 4	252	
8	280	
12	315	
15	335	Morphine sulphate, 251.25 mgms., i. e., 0.75 mgm. per gm. pig. Died 3½ hours.
April 4	240	
8	275	
12	290	
14	307	Morphine sulphate, 245.6 mgms., i. e., 0.8 mgm. Died 2 hours.

Summary. The results of the above experiments may be summarized as follows:

	Gm. thyroid fed daily.	Percentage of iodine in thyroid.	Mgm. Ī in thyroid fed daily.	Fatal dose in mgm. per gm.		Average loss of weight in per cent of original weight.[a]
				Recovered.	Died.	
Series I	0.12	0.084	0.1008	0.2	0.25	7.9
Controls				.5	.60	+21.2
Series II	.24	.084	.2016		.25	13.1
Controls				.65	.75	+27.2

[a] The + sign indicates that the guinea-pigs had increased in weight.

The guinea pigs which had received the thyroid died from one-third to one-half of the dose of morphine fatal to the controls. The amount of thyroid fed was very large and the animals were evidently deeply intoxicated.

Conclusions from the experiments with morphine.

The resistance of rats, mice, and guinea pigs to morphine is uniformly lowered by the feeding of thyroid. This occurs independently of any effect the thyroid may have upon the weight of the animals. In the case of the rat there is a close parallelism between the physiological effect of the thyroid, as determined by the increased susceptibility to morphine, and the percentage of iodine. A similar parallelism was found, in general, in the experiments on mice; the animals which had received thyroid with a higher percentage of iodine almost invariably died from a smaller dose of morphine than did those which had received thyroid with a low percentage of iodine. The question of the relation between the iodine content and the physiological activity of the thyroid was not tested on guinea pigs. The results of the experiments with morphine on rats and mice are therefore an additional argument for the view that the degree of physiological activity of thyroid is parallel to the iodine content.

We have no explanation to offer for the effect of thyroid feeding upon the resistance of animals to morphine further than the suggestion made by one of us in a previous paper:[a]

> I have not been able to determine the cause of this increased susceptibility possibly the thyroid, which is known to hasten the oxidation of fats, affects the lipoids of the nervous system in such a manner that poisons, such as morphine, gain acces to it more easily.

Mansfeld[b] found that rabbits which had been deprived of food for a few days were much more susceptible to morphine than were normal rabbits; he endeavored to explain this in the terms of the Meyer-Overton theory of narcosis: In starvation there is a diminution of fat in the body, except in the central nervous system; as a result of this a larger proportion of the fat soluble narcotic is taken up by the nervous system, and so is able to exert a greater toxic action. Although thyroid has a tendency to cause a diminution of fat in the body, we do not believe that this can have played a large part in our experiments: in some of these the thyroid had increased the susceptibility of the animals to morphine ten times without causing any change in weight.

These experiments may have some bearing upon the use of morphine in exophthalmic goiter (hyperthyroidism). There seems to be unanimity of opinion among clinicians that general anæsthetics are very dangerous in this condition.[c] The opinions differ as to the use of morphine or opium. Thus Tyson[a] considers opium to be a rational remedy, although he states that Ord and MacKenzie found that it is not well borne; Tyson prefers codeine. Our experiments showed that morphine is very toxic to animals in which a condition of thyroidism has been produced. Of course it has not been conclusively proved that there is a condition of hyperthyroidism in exophthalmic goiter or that man would react to morphine in the same way as do the lower animals. If we may judge of the degree of thyroidism from the loss of weight, our animals were as a rule certainly less deeply intoxicated than are many patients with exophthalmic goiter.

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[a] R. Hunt, J. Am. M. Ass., Chicago, 1907, **49,** p. 1325.

[b] G. Mansfeld, Arch. internat. de pharmacodynamie et de thérapie, 1905, **15,** p. 467

[c] *cf.* Th. Kocher, Verhandl. d. deutsch. Gesellsch. f. Chir., Berl., 1901, 30te Kong. p. 346; A. Kocher, Mitt. a. d. Grenzgeb. d. Med. u. Chir., Jena, 1902, **9,** p. 1. Angere (Münch. m. Wchschr., 1896, **43,** p. 71) states that the feeding of thyroid to patient with goiter renders them especially liable to heart failure when a general anæsthetic is administered.

The increased susceptibility to morphine of animals to which thyroid has been fed is suggestive in another connection.

May not many of the very diverse symptoms which occur in exophthalmic goiter be secondary, i. e., may not the condition of hyperthyroidism render many of the organs abnormally sensitive to poisons originating in the body itself (possibly products of intestinal putrefaction) or introduced from without (substances contained in meat, for example)? Organs rendered hypersensitive by the thyroid secretion may respond to amounts of such poisons ordinarily too small to produce any symptoms, just as the thyroid-fed rats may die from doses of morphine which would ordinarily be entirely innocuous.

As is well known there are few diseases in which so many drugs and forms of treatment have been recommended, often apparently with good reason, as in the case of exophthalmic goiter. Perhaps the effect of these is entirely secondary, i. e., some intestinal antiseptics[b] may check the formation of injurious substances, others may diminish the irritability of an over-stimulated organ.

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[a] J. Tyson, Internat. Clin., Phila., 1906, Series 16, **1,** p. 3.

[b] *cf.* W. H. Thomson, Am. J. Med. Sci., 1908, **135,** p. 313.

Part II.

ON THE NATURE OF THE RELATION BETWEEN THE IODINE PERCENTAGE AND THE PHYSIOLOGICAL ACTIVITY OF THYROID.

It has been shown above that there is almost invariably a parallelism between the iodine content of thyroid and its physiological activity. The nature of this relation may now be discussed. Some of the writers who recognize that there is such a parallelism hold that the larger amount of iodine in the more active thyroid is a result and not the cause of the greater activity. In other words, according to this view, the more active the gland the larger the amount of iodine with which its substance can combine.[a]

The arguments advanced for this theory are in part the same as those which have been urged against the view that the iodine is an mportant constituent of the thyroid and which have been discussed above. Such arguments are based largely upon what may be called statistical studies, and, as we have already seen, conclusions drawn from such studies do not necessarily apply to the thyroid when used as a drug. Moreover, were this view correct, we should expect to find at times thyroid poor in iodine but with a high degree of physiological activity, for the amount of iodine present in the thyroid is to a considerable extent accidental, being dependent upon the amount of this element available in the food. We have, however, never found a specimen of thyroid with a low percentage of iodine and with a high degree of physiological activity. Whether from the same or from widely separated species, the activity was in general parallel with the odine content.

A stronger argument against the view that the activity of thyroid s independent of the percentage of iodine is found in a metabolism experiment of Roos,[b] in which the activity of normal thyroid was compared with that of thyroid which had combined with a larger amount of iodine *in vivo*. Roos found the iodine-free thyroid of a dog to be without appreciable effect upon nitrogen excretion, whereas an equal amount of thyroid from a dog which had received 20 grams of potassium iodide in eighteen days and which contained 0.35 per cent iodine had a very distinct effect. Only one experiment was performed, and the result, although distinct, was not very marked.[c]

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[a] *cf.* S. J. Meltzer, N. Yorker med. Monatschr., 1907, **19**, p. 223.

[b] E. Roos, Ztschr. f. physiol. Chem., Strassb., 1899, **28**, p. 47.

[c] *cf.* F. Blum, Verhandl. d. Kong. f. inn. Med., 1906, **23**, p. 196.

Moreover, the evidence is far from conclusive that there may not have been differences between the two thyroid preparations other than that of the iodine content. There is also the possibility to be considered that the potassium iodide that was administered during the course of eighteen days had in some way simply stimulated the thyroid to an increased production of an active substance and that this active substance secondarily retained an additional amount of iodine and that the increased activity of the gland was due to the former and not to the latter.

It seemed to us that the following conditions should be fulfilled in order to make an experiment of this character conclusive: There should be evidence that the thyroid of the animals which received the iodine compound was, before its administration, in approximately the same condition as those which were to serve as controls; and, in the second place, the iodine should be administered for but a short time, so as to exclude any prolonged stimulating action. We endeavored to meet these conditions as follows: The thyroids of several groups of ten dogs each were analyzed for iodine, so as to obtain data as to the usual percentage of iodine present. To similar groups of ten dogs two doses of potassium iodide or of iodoform were given on successive days and the dogs killed on the third or fourth day. It was found that a large amount of iodine was taken up by the thyroid and that the latter, tested by our methods, showed greatly increased activity. Moreover, the increased activity was in direct proportion to the amount of iodine which had been taken up by the thyroid. We believe the conclusion is justified that the physiological activity of the thyroid from the different groups of dogs was approximately the same before the iodine was given, and therefore the increased activity of the thyroid of the iodine-fed dogs must have been due entirely to the iodine absorbed. The increased activity appeared much too rapidly to permit of the supposition that the iodine had simply accelerated the production of a more powerful substance independent of the iodine. The experiments were as follows:

SERIES A.

Forty-six dogs were divided into five groups. There were ten animals in each of the first three groups, seven in the fourth, and nine in the fifth. To each of the dogs of the fourth group from 1.5 to 2 grams of potassium iodide were given *per os;* they were killed twenty-four hours later. Each of the dogs of the fifth group received 1 to 1.3 grams iodoform in capsules; this was repeated on the following day and the dogs killed on the third day. The thyroids from all these dogs were weighed, ground up finely, dried at 50° C. and again weighed, and the iodine determined.

Group.	Weight of thyroid fresh.	Weight of thyroid dried.	Per cent of water.	Per cent of iodine.
	18.03	4.84	73.15	0.129
	25.67	6.86	73.27	.111
	14.09	3.99	71.68	.106
(fed KI)	13.25	3.35	74.71	.148
(fed CHI_3)	25.92	7.22	72.14	.30

The glands of the controls contained from 0.106 to 0.129 per cent odine; those which had received the potassium iodide had 0.148 and hose which had received the iodoform 0.3 per cent. The glands of ll five series lost approximately the same per cent of moisture on rying; this is additional evidence that they are strictly comparable.

The following experiments were performed with these preparations:

1. EXPERIMENTS WITH ACETONITRILE.

a. EXPERIMENTS ON MICE.

SERIES I.

A. THYROID OF GROUP 2 (NORMAL); 0.111 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1907.		
ne 5	12.05	Feeding of cakes each containing 0.0025 gms. thyroid commenced.
8	12.92	
11	12.92	
15	13.07	
16	12.63	Acetonitrile, 15.16 mgms., i. e., 1.2 mgms. per gm. mouse. Survived.
ne 5	10.73	Thyroid as above.
8	11.07	
11	10.85	
15	10.50	
16	10.75	Acetonitrile, 15.05 mgms., i. e., 1.4 mgms. per gm. mouse. Survived.
ne 5	12.95	Thyroid as above.
8	13.42	
11	11.77	
15	10.41	Acetonitrile, 16.66 mgms., i. e., 1.6 mgms. per gm. mouse. Died 3 hours.
ne 5	14.22	Thyroid as above.
8	13.82	
11	13.00	
15	11.60	Acetonitrile, 23.2 mgms., i. e., 2 mgms. per gm. mouse. Died 1 hour.

B. THYROID OF GROUP 4 (POTASSIUM IODIDE); 0.148 PER CENT IODINE.

1907.		
ne 5	11.06	Feeding of cakes each containing 0.0025 gm. thyroid commenced.
8	12.27	
11	12.35	
15	12.00	Acetonitrile, 19.2 mgms., i. e., 1.6 mgms. per gm. mouse. Survived.
ne 5	11.64	Thyroid as above.
8	11.52	
11	11.52	
15	12.15	
16	11.80	Acetonitrile, 22.42 mgms., i. e., 1.9 mgms. per gm. mouse. Died 1¼ hours.
ne 5	12.74	Thyroid as above.
8	12.02	
11	11.62	
15	11.11	
16	10.94	Acetonitrile, 24.07 mgms., i. e., 2.2 mgms. per gm. mouse. Died 3¼ hours.

C. THYROID OF GROUP 5 (IODOFORM); 0.3 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1907.		
June 5	12.52	Feeding of cakes each containing 0.0025 gm. thyroid commenced.
8	13.70	
11	13.20	
14	12.89	Acetonitrile, 18.05 mgms., i. e., 1.4 mgms. per gm. mouse. Survived.
June 5	14.01	Thyroid as above.
8	14.90	
11	13.70	
15	12.30	Acetonitrile, 22.14 mgms., i. e., 1.8 mgms. per gm. mouse. Survived.
June 5	13.30	Thyroid as above.
8	13.13	
11	13.20	
15	12.18	
16	12.10	Acetonitrile, 30.25 mgms., i. e., 2.5 mgms. per gm. mouse. Survived.
June 5	11.79	Thyroid as above.
8	11.83	
11	11.40	
15	10.18	
16	9.23	
17	8.71	Acetonitrile, 24.39 mgms., i. e., 2.8 mgms. per gm. mouse. Died 2 hours.

D. CONTROLS.

1907.		
June 5	12.48	Feeding of cakes without thyroid commenced.
8	13.61	
11	13.42	
15	12.96	Acetonitrile, 4.28 mgms., i. e., 0.33 mgm. per gm. mouse. Survived.
June 5	8.23	Cakes as above.
8	7.56	
11	8.07	
14	8.05	Acetonitrile, 2.98 mgms., i. e., 0.37 mgm. per gm. mouse. Survived.
June 5	12.10	Cakes as above.
8	13.16	
11	12.97	
14	13.00	Acetonitrile, 5.2 mgms., i. e., 0.4 mgm. per gm. mouse. Died about 2 hours.
June 5	12.30	Cakes as above.
8	11.83	
11	12.57	
15	12.66	
16	12.22	Acetonitrile, 4.89 mgms., i. e., 0.4 mgm. per gm. mouse. Died 2 hours.

Summary.—The results of the above experiments may be tabulated as follows:

	Gm. thyroid fed daily.	Percentage of iodine in thyroid.	Mgm. I in thyroid fed daily.	Fatal dose of acetonitrile in mgms. per gm.	
				Recovered.	Died.
A	0.0025	0.111	0.0027	1.4	1.6
B	.0025	.148	.0037	1.6	1.9
C	.0025	.300	.0075	2.5	2.8
D. Controls				.37	.40

From the above experiments it is seen that there is a direct parallelism between the per cent of iodine and the physiological activity of the thyroid. The thyroid of Group 5 (C) (from the dogs which had received iodoform and which contained 0.3 per cent iodine) enabled the mice to recover from about twice as much acetonitrile as did that from Group 2 (A) (normal, 0.111 per cent iodine). The thyroid of Group 4 (B) (potassium iodide fed dogs; 0.148 per cent iodine) was slightly more active than that of Group 2 and less active than that of Group 5. The amount of thyroid fed was comparatively large, and in the case of Group 5 was probably greater than the minimum amount necessary to produce the maximum effect.

SERIES II.

In the following experiments different amounts of the thyroid were fed; in the first three the amounts were selected so as to make the total iodine about the same in all.

A. THYROID OF GROUP 3 (NORMAL); 0.106 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1908.		
April 14	15.30	Feeding of cakes each containing 0.003 gm. thyroid commenced.
17	16.02	
20	16.37	
22	15.99	Acetonitrile, 34.38 mgms., i. e., 2.15 mgms. per gm. mouse. Survived.
April 14	21.60	Thyroid as above.
17	21.64	
20	21.34	
22	22.10	
23	22.03	Acetonitrile, 55.08 mgms., i. e., 2.5 mgms. per gm. mouse. Survived.
April 14	23.58	Thyroid as above.
17	24.74	
20	24.64	
23	25.05	Acetonitrile, 67.64 mgms., i. e., 2.7 mgms. per gm. mouse. Died one-half hour.
April 14	27.56	Thyroid as above.
17	27.72	
20	27.50	
23	28.30	Acetonitrile, 89.9 mgms., i. e., 3 mgms. per gm. mouse. Died 2 hours.

B. THYROID OF GROUP 4 (POTASSIUM IODIDE); 0.148 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1908.		
April 14	24.70	Feeding of cakes each containing 0.002 gm. thyroid commenced.
17	23.62	
20	23.57	
22	22.70	Acetonitrile, 56.75 mgms., i. e., 2.5 mgms. per gm. mouse. Survived.
April 14	19.12	Thyroid as above.
17	19.58	
20	19.68	
23	19.52	Acetonitrile, 50.75 mgms., i. e., 2.6 mgms. per gm. mouse. Survived.
April 14	15.10	Thyroid as above.
17	16.14	
20	16.14	
23	15.82	Acetonitrile, 45.88 mgms., i. e., 2.9 mgms. per gm. mouse. Survived.
April 14	22.72	Thyroid as above.
17	24.32	
20	24.92	
22	25.32	
23	25.50	Acetonitrile, 73.95 mgms., i. e., 2.9 mgms. per gm. mouse. Died 1½ hours.

C. THYROID OF GROUP 5 (IODOFORM); 0.3 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1908.		
April 14	22.95	Feeding of cakes each containing 0.001 gm. thyroid commenced.
17	22.64	
20	22.20	
22	23.02	Acetonitrile, 46.04 mgms., i. e., 2 mgms. per gm. mouse. Survived.
April 14	13.76	Thyroid as above.
17	14.03	
20	12.58	
23	12.50	Acetonitrile, 37.5 mgms., i. e., 3 mgms. per gm. mouse. Survived.
April 14	16.66	Thyroid as above.
17	17.24	
20	17.49	
23	17.70	Acetonitrile, 51.33 mgms., i. e., 2.9 mgms. per gm. mouse. Died 2½ hours.
April 14	24.36	Thyroid as above.
17	23.27	
20	23.52	
22	24.03	
23	23.36	Acetonitrile, 74.75 mgms., i. e., 3.2 mgms. per gm. mouse. Died one-half hour.

D. THYROID OF GROUP 5 (IODOFORM); 0.3 PER CENT IODINE.

1908.		
April 14	19.36	Feeding of cakes each containing 0.002 gm. thyroid commenced.
17	19.70	
20	20.26	
22	20.74	Acetonitrile, 67.4 mgms., i. e., 3.25 mgms. per gm. mouse. Survived.
April 14	17.75	Thyroid as above.
17	18.85	
20	18.57	
22	18.42	
23	18.44	Acetonitrile, 73.76 mgms., i. e., 4 mgms. per gm. mouse. Survived.
April 14	14.94	Thyroid as above.
17	15.36	
20	15.28	
23	15.33	Acetonitrile, 67.45 mgms., i. e., 4.4 mgms. per gm. mouse. Survived.
April 14	24.09	Thyroid as above.
17	26.07	
20	25.45	
23	25.33	
24	25.15	Acetonitrile, 125.75 mgms., i. e., 5 mgms. per gm. mouse. Died 2 hours.

E. CONTROLS.

1908.		
April 14	17.56	Feeding of cakes without thyroid commenced.
17	17.76	
20	16.42	
23	16.71	Acetonitrile, 10.86 mgms., i. e., 0.65 mgm. per gm. mouse. Survived.
April 14	27.94	Cakes as above.
17	28.76	
20	28.58	
23	27.83	Acetonitrile, 19.94 mgms., i. e., 0.72 mgm. per gm. mouse. Survived.
April 14	19.90	Cakes as above.
17	19.62	
20	19.34	
22	19.90	
23	19.44	Acetonitrile, 14.58 mgms., i. e., 0.75 mgm. per gm. mouse. Died 2 hours.
April 14	23.54	Cakes as above.
17	23.86	
20	23.92	
23	23.54	
24	23.04	Acetonitrile, 17.74 mgms., i. e., 0.77 mgm. per gm. mouse. Died 1¾ hours.

Summary.—The results of the foregoing experiments may be tabu-
ated as follows:

	Gm. thyroid fed daily.	Percentage of iodine in thyroid.	Mgm. I in thyroid fed daily.	Fatal dose of acetonitrile in mgms. per gm.	
				Recovered.	Died.
	0.003	0.106	0.00318	2.5	2.7
	.002	.148	.00296	2.9	2.9
	.001	.300	.0030	3.0	2.9
	.002	.300	.0060	4.4	5.0
Controls				.72	.75

Thyroid containing 0.106, 0.148, and 0.3 per cent iodine, respec-
ively, was fed to mice. When fed in amounts corresponding to the
atio of 3, 2, and 1 (namely, in the inverse ratio to the iodine content)
he physiological effects were approximately equal. With equal
mounts of the thyroid containing 0.148 and 0.3 per cent iodine the
atter was much the more active.

SERIES III.

In the following experiments the effects of the thyroid of Group 3
normal; 0.106 per cent iodine) and of Group 5 (iodoform fed dogs;
.3 per cent iodine) were compared.

A. THYROID OF GROUP 3 (NORMAL); 0.106 PER CENT IODINE.

Date.	Weight.	Remarks.
1908.		
pril 25	18.45	Feeding of cakes each containing 0.0005 gm. thyroid commenced.
29	19.74	
ay 2	19.74	
3	19.74	Acetonitrile, 35.53 mgms., i. e., 1.8 mgms. per gm. mouse. Survived.
pril 25	15.00	Thyroid as above.
29	15.16	
ay 2	15.14	
4	15.28	
5		Acetonitrile, 29.03 mgms., i. e., 1.9 mgms. per gm. mouse. Survived.
pril 25	15.73	Thyroid as above.
29	14.88	
ay 2	14.50	
4	14.34	Acetonitrile, 30.11 mgms., i. e., 2.1 mgms. per gm. mouse. Died 6 hours.
pril 25	16.87	Thyroid as above.
29	18.20	
ay 2	17.60	
4	18.15	Acetonitrile, 41.75 mgms., i. e., 2.3 mgms. per gm. mouse. Died 3½ hours.

B. THYROID OF GROUP 3 (NORMAL); 0.106 PER CENT IODINE.

Date.	Weight.	Remarks.
1908.		
April 25	13.75	Feeding of cakes each containing 0.0015 gm. thyroid commenced.
29	14.34	
May 2	14.92	
3	14.92	Acetonitrile, 37.3 mgms., i. e., 2.5 mgms. per gm. mouse. Survived.
April 25	14.20	Thyroid as above.
29	14.30	
May 2	13.48	
4	13.28	Acetonitrile, 41.17 mgms., i. e., 3.1 mgms. per gm. mouse. Survived.
April 25	16.32	Thyroid as above.
29	15.28	
May 2	15.32	
4	14.68	
5		Acetonitrile, 52.85 mgms., i. e., 3.6 mgms. per gm. mouse. Died 1½ hours.
April 25	16.23	Thyroid as above.
29	17.68	
May 2	17.34	
4	16.28	Acetonitrile, 61.86 mgms., i. e., 3.8 mgms. per gm. mouse. Died 50 hours.

C. THYROID OF GROUP 5 (IODOFORM FED DOGS); 0.3 PER CENT IODINE.

1908.		
April 25	14.93	Feeding of cakes each containing 0.0005 gm. thyroid commenced.
29	16.50	
May 2	16.84	
3	16.84	Acetonitrile, 45.47 mgms., i. e., 2.7 mgms. per gm. mouse. Survived.
April 25	15.99	Thyroid as above.
29	16.64	
May 2	16.32	
4	16.54	Acetonitrile, 51.27 mgms., i. e., 3.1 mgms. per gm. mouse. Survived.
April 25	15.49	Thyroid as above.
29	16.37	
May 2	12.96	
4	14.25	Acetonitrile, 52.72 mgms., i. e., 3.7 mgms. per gm. mouse. Survived.
April 25	15.13	Thyroid as above.
29	15.64	
May 2	14.75	
4	14.72	
5		Acetonitrile, 61.82 mgms., i. e., 4.2 mgms. per gm. mouse. Died 1½ hours.

D. THYROID OF GROUP 5.

1908.		
April 25	15.05	Feeding of cakes each containing 0.001 gm. thyroid commenced.
29	16.08	
May 2	15.66	
3	15.66	Acetonitrile, 46.98 mgms., i. e., 3 mgms. per gm. mouse. Survived.
April 25	14.84	Thyroid as above.
29	15.44	
May 2	14.24	
4	14.15	Acetonitrile, 50.94 mgms., i. e., 3.6 mgms. per gm. mouse. Survived
April 25	15.24	Thyroid as above.
29	15.00	
May 2	14.83	
4	14.69	Acetonitrile, 60.23 mgms., i. e., 4.1 mgms. per gm. mouse. Survived.
April 25	15.51	Thyroid as above.
29	16.04	
May 2	16.16	
4	15.80	
5		Acetonitrile, 74.26 mgms., i. e., 4.7 mgms. per gm. mouse. Died 4½ hours.

E. CONTROLS.

Date.	Weight.	Remarks.
1908.		
April 25	13.45	Feeding of cakes without thyroid commenced.
29	14.47	
May 2	14.56	
3	14.56	Acetonitrile, 7.43 mgms., i. e., 0.51 mgm. per gm. mouse. Survived.
April 25	18.43	Cakes as above.
29	19.67	
May 2	19.76	
4	19.54	
5	....	Acetonitrile, 10.16 mgms., i. e., 0.52 mgm. per gm. mouse. Died 1 hour.
April 25	16.88	Cakes as above.
29	15.43	
May 2	15.57	
4	15.21	
5	....	Acetonitrile, 8.06 mgms., i. e., 0.53 mgm. per gm. mouse. Died 8 hours.

Summary.—The results of the above experiments may be tabulated as follows:

	Gm. thyroid fed daily.	Percentage of iodine in thyroid.	Mgm. I in thyroid fed daily.	Fatal dose of acetonitrile in mgms. per gm.	
				Recovered.	Died.
A	0.0005	0.106	0.00053	1.9	2.1
B	.0015	.106	.00159	3.1	3.6
C	.0005	.300	.00150	3.7	4.2
D	.001	.300	.00300	4.1	4.7
E. Controls	....	....	....	.51	.52

With equal amounts of thyroid containing, respectively, 0.106 and 0.3 per cent iodine, the effect of the latter was much greater than that of the former; with three parts of the former and one part of the latter the effects were nearly equal. One part of the thyroid rich in iodine was also much more active than one and one-half parts of the thyroid poor in iodine.

SERIES IV.

In this series equal amounts of the thyroid of Groups 2 and 1 (normal) and of Group 5 (iodoform fed dogs) were compared.

A. THYROID OF GROUP 2 (NORMAL); 0.111 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1908.		
May 9	13.83	Feeding of cakes, each containing 0.0005 gm. thyroid, commenced.
16	12.49	
18	11.76	Acetonitrile, 12.94 mgms., i. e., 1.1 mgms. per gm. mouse. Survived.
May 9	18.52	Thyroid as above.
16	18.43	
18	18.29	Acetonitrile, 26.61 mgms., i. e., 1.4 mgms. per gm. mouse. Died 5 hours.
May 9	18.48	Thyroid as above.
16	19.00	
17		Acetonitrile, 34.2 mgms., i. e., 1.8 mgms. per gm. mouse. Died 2 hours.
May 9	14.98	Thyroid as above.
16	12.80	
17		Acetonitrile, 32.0 mgms., i. e., 2.5 mgms. per gm. mouse. Died 3 hours.

B. THYROID OF GROUP 1 (NORMAL); 0.129 PER CENT IODINE.

1908.		
May 9	16.17	Feeding of cakes, each containing 0.0005 gm. thyroid, commenced.
15	16.84	
18	16.71	Acetonitrile, 26.74 mgms., i. e., 1.6 mgms. per gm. mouse. Survived.
May 9	11.50	Thyroid as above.
15	12.67	
16	12.46	
18	12.59	Acetonitrile, 22.62 mgms., i. e., 1.8 mgms. per gm. mouse. Survived.
May 9	15.87	Thyroid as above.
15	15.17	
16	14.87	
17		Acetonitrile, 31.23 mgms., i. e., 2.1 mgms. per gm. mouse. Died 1¼ hours.
May 9	15.71	Thyroid as above.
15	14.61	
16	14.58	
17		Acetonitrile, 36.45 mgms., i. e., 2.5 mgms. per gm. mouse. Died 1½ hours.

C. THYROID OF GROUP 5 (IODOFORM); 0.3 PER CENT IODINE.

1908.		
May 9	21.46	Feeding of cakes, each containing 0.0005 gm. thyroid, commenced.
16	19.13	
18	19.15	Acetonitrile, 55.54 mgms., i. e., 2.9 mgms. per gm. mouse. Survived.
May 9	14.50	Thyroid as above.
16	13.39	
17		Acetonitrile, 41.51 mgms., i. e., 3.1 mgms. per gm. mouse. Survived..
May 9	16.12	Thyroid as above.
16	15.02	
18	15.05	Acetonitrile, 49.66 mgms., i. e., 3.3 mgms. per gm. mouse. Died 2½ hours.
May 9	14.26	Thyroid as above.
16	12.25	Acetonitrile, 42.88 mgms., i. e., 3.5 mgms. per gm. mouse. Died 4⅓ hours.

D. (CONTROLS.)

Date.	Weight of mouse.	Remarks.
1908.		
May 9	16.65	Feeding of cakes without thyroid commenced.
15	16.06	
16	16.05	
17		Acetonitrile, 5.78 mgms., i. e., 0.36 mgm. per gm. mouse. Survived.
May 9	16.50	Cakes as above.
15	15.74	
16	15.42	
18	15.56	Acetonitrile, 5.76 mgms., i. e., 0.37 mgm. per gm. mouse. Died 2 hours.
May 9	15.79	Cakes as above.
15	11.80	
16	12.15	
18	13.16	Acetonitrile, 5.27 mgms., i. e. 0.4 mgm. per gm. mouse. Died 4 hours.

Summary.—The results of the above experiments may be summarized as follows:

	Gm. thyroid fed daily.	Percentage of iodine in thyroid.	Mgm. I in thyroid fed daily.	Fatal dose of acetonitrile in mgms. per gm.	
				Recovered.	Died.
A	0.0005	0.111	0.00055	1.1	1.4
B	.0005	.129	.00064	1.8	2.1
C	.0005	.300	.00150	3.1	3.3
D. Controls				.36	.37

The thyroid of the dogs which had received iodoform was much more active than that of the normal dogs.

b. Experiments on Rats.

Results very similar to the above were obtained in a series of experiments upon rats. In these animals there is a diminution of resistance to acetonitrile.

A. THYROID OF GROUP 2 (NORMAL); 0.111 PER CENT IODINE.

Date.	Weight of rat.	Remarks.
1908.		
May 15	32	Feeding of cakes, each containing 0.0005 gm. thyroid, commenced.
18	33	
20	33	
23	32	Acetonitrile, 51.2 mgms., i. e., 1.6 mgms. per gm. rat. Died about 17 hours.
May 15	43	Thyroid as above.
18	43	
20	43	
23	41	
24		Acetonitrile, 73.8 mgms., i. e., 1.8 mgms. per gm. rat. Survived.
May 15	50	Thyroid as above.
18	50	
20	49	
23	48	Acetonitrile, 100.8 mgms., i. e., 2.1 mgms. per gm. rat. Died 2 hours.
May 15	35	Thyroid as above.
18	34	
20	34	
23	32	
25	32	Acetonitrile, 70.4 mgms., i. e., 2.2 mgms. per gm. rat. Died 4 hours.

B. THYROID OF GROUP 5 (IODOFORM); 0.3 PER CENT IODINE.

Date.	Weight of rat.	Remarks.
1908.		
May 15	36	Feeding of cakes, each containing 0.0005 gm. thyroid, commenced.
18	37	
20	37	
23	34	Acetonitrile, 34 mgms., i. e., 1 mgm. per gm. rat. Survived.
May 15	44	Thyroid as above.
18	42	
20	43	
23	41	
24	41	Acetonitrile, 53.3 mgms., i. e., 1.3 mgms. per gm. rat. Survived.
May 15	38	Thyroid as above.
18	37	
20	36	
23	35	Acetonitrile, 52.5 mgms., i. e., 1.5 mgms. per gm. rat. Died 1½ hours.

C. THYROID OF GROUP 2 (NORMAL); 0.111 PER CENT IODINE.

1908.		
May 15	41	Feeding of cakes, each containing 0.0015 gm. thyroid, commenced.
18	39	
20	38	
23	38	Acetonitrile, 45.6 mgms., i. e., 1.2 mgms. per gm. rat. Survived.
May 15	43	Thyroid as above.
18	41	
20	41	
23	38	
24	....	Acetonitrile, 49.4 mgms., i. e., 1.3 mgms. per gm. rat. Died 1 hour.
May 15	36	Thyroid as above.
18	35	
20	34	
23	33	
24	....	Acetonitrile, 46.2 mgms., i. e., 1.4 mgms. per gm. rat. Died 4 to 7 hours.
May 15	64	Thyroid as above.
18	57	
20	59	
23	58	Acetonitrile, 92.8 mgms., i. e., 1.6 mgms. per gm. rat. Died 3 hours.

D. THYROID OF GROUP 2 (NORMAL); 0.111 PER CENT IODINE.

1908.		
May 15	38	Feeding of cakes, each containing 0.003 gm. thyroid, commenced.
18	37	
20	36	
23	35	Acetonitrile, 28 mgms., i. e., 0.8 mgm. per gm. rat. Survived.
May 15	45	Thyroid as above.
18	43	
20	43	
23	41	
24	41	Acetonitrile 36.9 mgms., i. e., 0.9 mgm. per gm. rat. Survived.
May 15	37	Thyroid as above.
18	35	
20	37	
23	34	
24	....	Acetonitrile, 34 mgms., i. e., 1 mgm. per gm. rat. Died 1 hour.
May 15	31	Thyroid as above.
18	31	
20	31	
23	29	Acetonitrile, 31.9 mgms., i. e., 1.1 mgms. per gm. rat. Died 2½ hours.

E. THYROID OF GROUP 5 (IODOFORM); 0.3 PER CENT IODINE.

Date.	Weight of rat.	Remarks.
1908.		
May 15	43	Feeding of cakes, each containing 0.001 gm. thyroid, commenced.
18	36	
20	37	
23	36	
24	36	Acetonitrile, 18 mgms., i. e., 0.5 mgm. per gm. rat. Survived.
May 15	38	Thyroid as above.
18	37	
20	38	
23	35	
24	....	Acetonitrile, 21 mgms., i. e., 0.6 mgm. per gm. rat. Died 1 to 2 hours.
May 15	36	Thyroid as above.
18	34	
20	35	
23	33	Acetonitrile, 23.1 mgms., i. e., 0.7 mgm. per gm. rat. Died 4½ hours.
May 15	41	Thyroid as above.
18	38	
20	38	
23	35	Acetonitrile, 35 mgms., i. e., 1 mgm. per gm. rat. Died 2¼ hours.

F. CONTROLS.

Date.	Weight of rat.	Remarks.
1908.		
May 15	41	Feeding of cakes without thyroid commenced.
18	38	
20	37	
23	38	Acetonitrile, 87.4 mgms., i. e., 2.3 mgms. per gm. rat. Survived.
May 15	41	Cakes as above.
18	39	
20	37	
23	36	Acetonitrile, 100.8 mgms., i. e., 2.8 mgms. per gm. rat. Survived.
May 15	50	Cakes as above.
18	46	
20	48	
23	44	
24	....	Acetonitrile, 127.6 mgms. i. e., 2.9 mgms. per gm. rat. Survived.
May 15	69	Cakes as above.
18	64	
20	65	
23	62	
24	....	Acetonitrile, 186 mgms., i. e., 3 mgms. per gm. rat. Died 2½ hours.
May 15	42	Cakes as above.
18	38	
20	38	
23	37	Acetonitrile, 114.7 mgms., i. e., 3.1 mgms. per gm. rat. Died 3 to 6 hours.

Summary.—The results of the above experiments may be tabulated as follows:

	Gm. thyroid in each cake.	Percentage of iodine in thyroid.	Mm. I in thyroid of each cake.	Fatal dose of acetonitrile in mgms. per gm.	
				Recovered.	Died.
A	0.0005	0.111	0.00055	1.8	2.1
B	.0005	.300	.00150	1.3	1.5
C	.0015	.111	.00166	1.2	1.3
D	.003	.111	.00333	.9	1.0
E	.001	.300	.00300	.5	.6
F. Controls	....	....	....	2.9	3.0

With equal amounts of thyroid that containing the higher percentage of iodine was the more active. To obtain approximately equal effects it was necessary to give three times as much of the thyroid poor in iodine as of the thyroid rich in iodine.

2. EXPERIMENTS WITH MORPHINE.

a. Experiments on Mice.

Only one short series of experiments was performed in which the effect of feeding the above thyroid preparations upon the resistance of mice to morphine sulphate was tested.

The results indicate that the thyroid of the iodoform-fed dogs is distinctly more active in lowering the resistance of mice to morphine than is that of the normal dogs.

A. THYROID OF GROUP 2 (NORMAL); 0.111 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1907.		
July 3	11.64	Feeding of cakes, each containing 0.01 gm. thyroid, commenced.
8	11.74	
11	11.10	
13	10.40	Morphine sulphate, 0.73 mgms., i. e., 0.07 mgm. per gm. mouse. Survived
July 3	13.33	Thyroid as above.
8	13.64	
11	14.45	
14	13.33	Morphine sulphate, 1.47 mgms., i. e., 0.11 mgm. per gm. mouse. Survived.
July 3	12.14	Thyroid as above.
8	12.77	
11	12.05	
13	11.25	Morphine sulphate, 1.8 mgms., i. e., 0.16 mgm. per gm. mouse. Died three-fourths hour.

B. THYROID OF GROUP 5 (IODOFORM); 0.3 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1907.		
July 3	14.65	Feeding of cakes, each containing 0.01 gm. thyroid, commenced.
8	16.22	
12	13.00	
14	11.76	Morphine sulphate, 0.82 mgm., i. e., 0.07 mgm. per gm. mouse. Survived.
July 3	12.92	Thyroid as above.
8	13.02	
12	13.32	
14	11.94	Morphine sulphate, 1.07 mgms., i. e., 0.09 mgm. per gm. mouse. Survived.
July 3	13.23	Thyroid as above.
8	13.10	
12	12.45	
13	12.32	Morphine sulphate, 1.23 mgms., i. e., 0.1 mgm. per gm. mouse. Died 2 hours

C. CONTROLS.

Date.	Weight of mouse.	Remarks.
1907.		
July 3	13.30	Feeding of cakes without thyroid commenced.
8	14.40	
12	14.25	Morphine sulphate, 5.27 mgms., i. e., 0.37 mgm. per gm. mouse. Survived.
July 3	11.40	Cakes as above.
8	11.90	
12	12.45	
13	12.33	Morphine sulphate, 5.18 mgms., i. e., 0.42 mgm. per gm. mouse. Survived.
July 3	14.25	Cakes as above.
8	14.46	
12	14.85	
14	14.40	Morphine sulphate, 6.19 mgms., i. e., 0.43 mgm. per gm. mouse. Survived.
July 3	12.90	Cakes as above.
8	15.32	
12	15.72	
15	15.40	Morphine sulphate, 6.93 mgms., i. e., 0.45 mgm. per gm. mouse. Died 1¼ hours
July 3	13.85	Cakes as above.
8	12.90	
12	11.77	
14	11.75	Morphine sulphate, 5.4 mgms., i. e., 0.46 mgm. per gm. mouse. Died about 2 hours.

Summary.—The results of these experiments may be tabulated as follows:

	Gm. thyroid fed daily.	Percentage of iodine in thyroid.	Mg. I in thyroid fed daily.	Fatal dose of morphine sulphate in mgm. per gm.	
				Recovered.	Died.
A	0.01	0.111	0.0111	0.11	0.16
B	.01	.300	.0300	.09	.10
C. Controls				.43	.45

b. EXPERIMENTS ON RATS.

The following series of experiments on rats, although very incomplete, show that the activity of the thyroid, the iodine content of which has been increased by the administration of iodoform to dogs, was much greater than that of normal thyroid.

SERIES I.

A. THYROID OF GROUP 2 (NORMAL); 0.111 PER CENT IODINE.

Date.	Weight of rat.	Remarks.
1908.		
May 15	43	Feeding of cakes, each containing 0.0005 gm. thyroid, commenced.
18	43	
20	43	
23	41	
27	40	Morphine sulphate, 10 mgms., i. e., 0.25 mgm. per gm. rat. Survived.

B. THYROID OF GROUP 5 (IODOFORM); 0.3 PER CENT IODINE.

Date.	Weight of rat.	Remarks.
1908.		
May 15	36	Feeding of cakes, each containing 0.0005 gm. thyroid, commenced.
18	37	
20	37	
23	34	
25	34	Morphine sulphate, 4.76 mgms., i. e., 0.14 mgm. per gm. rat. Survived.
May 15	41	Thyroid as above.
18	39	
20	40	
23	39	
26	35	Morphine sulphate, 7. mgms., i. e., 0.2 mgm. per gm. rat. Survived.
May 15	44	Thyroid as above.
18	42	
20	43	
23	41	
24	41	
28	43	Morphine sulphate, 10.75 mgms., i. e., 0.25 mgm. per gm. rat. Died 5 hours.

C. THYROID OF GROUP 2 (NORMAL); 0.111 PER CENT IODINE.

1908.		
May 15	41	Feeding of cakes, each containing 0.0015 gm. thyroid, commenced.
18	39	
20	38	
23	38	
27	40	Morphine sulphate, 10 mgms., i. e., 0.25 mgm. per gm. rat. Died 2¼ hours.

D. THYROID OF GROUP 2 (NORMAL); 0.111 PER CENT IODINE.

1908.		
May 15	38	Feeding of cakes, each containing 0.003 gm. thyroid, begun.
18	37	
20	36	
23	35	
26	40	Morphine sulphate, 6.4 mgms., i. e., 0.16 mgm. per gm. rat. Survived.
May 15	45	Thyroid as above.
18	43	
20	43	
23	41	
24	41	
28	43	Morphine sulphate, 8.6 mgms., i. e., 0.2 mgm. per gm. rat. Died 3½ hours.

E. THYROID OF GROUP 3 (NORMAL); 0.106 PER CENT IODINE.

1908.		
May 15	54	Feeding of cakes, each containing 0.001 gm. thyroid, commenced.
18	52	
20	51	
23	50	
24	50	
27	51	Morphine sulphate, 12.75 mgms., i. e., 0.25 mgm. per gm. rat. Survived.

F. THYROID OF GROUP 5 (IODOFORM); 0.3 PER CENT IODINE.

1908.		
May 15	43	Feeding of cakes, each containing 0.001 gm. thyroid, commenced.
18	36	
20	37	
23	36	
24	36	
27	37	Morphine sulphate, 7.4 mgms., i. e., 0.2 mgm. per gm. rat. Died 5 hours.

G. CONTROLS.

Date.	Weight of rat.	Remarks.
1908.		
May 15	41	Feeding of cakes without thyroid commenced.
18	38	
20	37	
23	38	
26	40	Morphine sulphate, 13.6 mgms., i. e., 0.34 mgm. per gm. rat. Survived.
May 15	41	Cakes as above.
18	39	
20	37	
23	36	
28	36	Morphine sulphate, 14 mgms., i. e., 0.39 mgm. per gm. rat. Survived.
May 15	50	Cakes as above.
18	46	
20	48	
23	44	
27	44	Morphine sulphate, 17.6 mgms., i. e., 0.4 mgm. per gm. rat. Died 4 hours.

Summary.—These experiments may be summarized as follows:

	Gm. thyroid in each cake.	Percentage of iodine in thyroid.	Mgm. I in thyroid of each cake.	Loss of weight in per cent of original weight.	Fatal dose of morphine sulphate in mgm. per gm.	
					Recovered.	Died.
A	0.0005	0.111	0.00055	7.0	0.25	....
B	.0005	.3	.0015	7.6	.20	0.25
C	.0015	.111	.00166	2.4	....	.25
D	.003	.111	.00333	.0	.16	.20
E	.001	.106	.00106	5.5	.25	....
F	.001	.3	.0030	14.0	....	.20
G. Controls	....	....	....	9.1	.39	.40

These experiments show that with equal amounts of thyroid containing different percentages of iodine those with the larger amounts of iodine caused the greater lowering of resistance to morphine; also that in order to obtain equal physiological effects it was necessary to feed much more of the iodine-poor thyroid. Loss of weight can not explain the increased susceptibility of the rats to morphine, for with but one exception there was no loss of weight greater than that which could be ascribed to the exclusive cracker diet.

SERIES B.

Twenty-three dogs were divided into three groups. There were six dogs in the first group (Group 6), nine in the second (Group 7), and eight in the third (Group 8). Each of the dogs of Group 6 received about 1.2 grams iodoform in capsules on two successive days; they were killed on the day following that on which they had received the second dose. The glands were weighed and dried as described under Series A.

Group.	Weight of thyroid fresh.	Weight of thyroid dried.	Per cent of water.	Per cent of iodine.
6 (iodoform)	12.40	3.20	74.2	0.21
7 (normal)	33.05	8.69	73.7	.10
8 (normal)	17.40	4.60	73.5	.12

The following experiments were performed with these thyroids and some of those of Series A.

A. THYROID OF GROUP 8 (NORMAL); 0.12 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1908.		
July 13	19.13	Feeding of cakes, each containing 0.001 gm. thyroid, commenced.
17	17.18	
21	17.80	Acetonitrile, 8.01 mgms., i. e., 0.45 mgm. per gm. mouse. Survived.
July 13	16.40	Thyroid as above.
17	15.89	
21	15.66	Acetonitrile, 8.61 mgms., i. e., 0.55 mgm. per gm. mouse. Died 1 hour.
July 13	14.51	Thyroid as above.
17	14.09	
21	12.73	Acetonitrile, 8.91 mgms., i. e., 0.7 mgm. per gm. mouse. Died 2½ hours.

B. THYROID OF GROUP 2 (NORMAL); 0.111 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1908.		
July 13	15.36	Feeding of cakes, each containing 0.001 gm. thyroid, commenced.
17	13.78	
21	12.45	Acetonitrile, 8.09 mgms., i. e., 0.65 mgm. per gm. mouse. Died about 4 hours.
July 13	12.81	Thyroid as above.
17	11.59	
21	11.25	Acetonitrile, 10.13 mgms., i. e., 0.9 mgm. per gm. mouse. Died 1 hour.

C. THYROID OF GROUP 6 (IODOFORM); 0.21 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1908.		
July 13	14.00	Feeding of cakes, each containing 0.001 gm. thyroid, commenced.
17	13.25	
21	12.17	Acetonitrile, 10.95 mgms., i. e., 0.9 mgm. per gm. mouse. Survived.
July 13	11.80	Thyroid as above.
17	10.59	
21	11.29	Acetonitrile, 15.81 mgms., i. e., 1.4 mgms. per gm. mouse. Survived.
July 13	13.78	Thyroid as above.
17	14.14	
21	14.30	Acetonitrile, 22.88 mgms., i. e., 1.6 mgms. per gm. mouse. Survived.
July 13	12.25	Thyroid as above.
17	12.36	
21	9.84	Acetonitrile, 18.70 mgms., i. e., 1.9 mgms. per gm. mouse. Died 1 hour.

D. THYROID OF GROUP 5 (IODOFORM); 0.3 PER CENT IODINE.

Date.	Weight of mouse.	Remarks.
1908.		
July 13	15.91	Feeding of cakes, each containing 0.001 gm. thyroid, commenced.
17	14.88	
21	14.90	Acetonitrile, 19.37 mgms., i. e., 1.3 mgms. per gm. mouse. Survived.
July 13	17.39	Thyroid as above.
17	16.33	
21	16.20	Acetonitrile, 30.78 mgms., i. e., 1.9 mgms. per gm. mouse. Survived.
July 13	14.52	Thyroid as above.
17	13.72	
21	12.63	
22		Acetonitrile, 26.52 mgms., i. e., 2.1 mgms. per gm. mouse. Survived.
July 13	17.78	Thyroid as above.
17	16.40	
21	17.12	Acetonitrile, 41.09 mgms., i. e., 2.4 mgms. per gm. mouse. Died 3 hours.

E. CONTROLS.

1908.		
July 13	14.35	Feeding of cakes without thyroid commenced.
17	13.98	
21	13.53	Acetonitrile, 3.25 mgms., i. e., 0.24 mgm. per gm. mouse. Survived.
July 13	13.25	Cakes as above.
17	12.83	
21	12.96	Acetonitrile, 3.24 mgms., i. e., 0.25 mgm. per gm. mouse. Died 1 hour.
July 13	14.20	Cakes as above.
17	13.03	
21	11.50	
22		Acetonitrile, 2.99 mgms., i. e., 0.26 mgm. per gm. mouse. Died 2½ hours.

SUMMARY.

	Gm. thyroid fed daily.	Percentage of iodine in thyroid.	Mgm. I in thyroid fed daily.	Fatal dose of acetonitrile in mgms. per gm.	
				Recovered.	Died.
A	0.001	0.12	0.0012	0.45	0.55
B	.001	.111	.00111		.65
C	.001	.21	.0021	1.6	1.9
D	.001	.3	.0030	2.1	2.4
E, Controls				.24	.25

The results are very similar to those obtained with the thyroid of Series A. The thyroid, the iodine content of which had been increased by the administration of iodoform, was much more active in protecting mice against acetonitrile than was that of the normal dogs.

GENERAL SUMMARY OF ABOVE EXPERIMENTS AND CONCLUSIONS.

The experiments described above show that when potassium iodide or iodoform is administered to dogs the thyroids of the latter contain a greatly increased percentage of iodine and also are much more active physiologically. This result occurred too quickly to admit of any explanation other than that the increased activity was due to the increased iodine content. It seems to us to show conclusively that thyroid rich in iodine is more active than thyroid poor in iodine simply on account of the iodine; in other words, that the iodine is the cause and not the result of the activity.

LIST OF HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH AND MARINE-HOSPITAL SERVICE.

The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, located in Washington, was authorized by act of Congress, March 3, 1901.

The following *bulletins* [Bulls. Nos. 1-7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.] have been issued:

*No. 1.—Preliminary note on the viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau.

*No. 3.—Sulphur dioxid as a germicidal agent. By H. D. Geddings.

*No. 4.—Viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 5.—An investigation of a pathogenic microbe (*B. typhi murium* Danyz) applied o the destruction of rats. By M. J. Rosenau.

*No. 6.—Disinfection against mosquitoes with formaldehyd and sulphur dioxid, By M. J. Rosenau.

No. 7.—Laboratory technique: Ring test for indol, by S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis; Microphotography with simple apparatus, by H. B. Parker.

By act of Congress approved July 1, 1902, the name of the "United States Marine-Hospital Service" was changed to the "Public Health and Marine-Hospital Service of he United States," and three new divisions were added to the Hygienic Laboratory.

Since the change of name of the Service the bulletins of the Hygienic Laboratory have been continued in the same numerical order, as follows:

*No. 8.—Laboratory course in pathology and bacteriology. By M. J. Rosenau. Revised edition, March, 1904.)

*No. 9.—Presence of tetanus in commercial gelatin. By John F. Anderson.

No. 10.—Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States. By Ch. Wardell Stiles.

*No. 11.—An experimental investigation of *Trypanosoma lewisi*. By Edward Francis.

*No. 12.—The bacteriological impurities of vaccine virus; an experimental study. By M. J. Rosenau.

*No. 13.—A statistical study of the intestinal parasites of 500 white male patients at he United States Government Hospital for the Insane; by Philip E. Garrison, Brayton H. Ransom, and Earle C. Stevenson. A parasitic roundworm (*Agamomermis ulicis* n. g., n. sp.) in American mosquitoes (*Culex sollicitans*); by Ch. Wardell Stiles. The type species of the cestode genus *Hymenolepis;* by Ch. Wardell Stiles.

No. 14.—Spotted fever (tick fever) of the Rocky Mountains; a new disease. By John F. Anderson.

No. 15.—Inefficiency of ferrous sulphate as an antiseptic and germicide. By Allan J. McLaughlin.

*No. 16.—The antiseptic and germicidal properties of glycerin. By M. J. Rosenau.

*No. 17.—Illustrated key to the trematode parasites of man. By Ch. Wardell Stiles.

*No. 18.—An account of the tapeworms of the genus *Hymenolepis* parasitic in man, including reports of several new cases of the dwarf tapeworm (*H. nana*) in the United States. By Brayton H. Ransom.

*No. 19.—A method for inoculating animals with precise amounts. By M. J. Rosenau.

*No. 20.—A zoological investigation into the cause, transmission, and source of Rocky Mountain "spotted fever." By Ch. Wardell Stiles.

No. 21.—The immunity unit for standardizing diphtheria antitoxin (based on Ehrlich's normal serum). Official standard prepared under the act approved July 1, 1902. By M. J. Rosenau.

*No. 22.—Chloride of zinc as a deodorant, antiseptic, and germicide. By T. B. McClintic.

*No. 23.—Changes in the Pharmacopœia of the United States of America. Eighth Decennial Revision. By Reid Hunt and Murray Galt Motter.

No. 24.—The International Code of Zoological Nomenclature as applied to medicine. By Ch. Wardell Stiles.

No. 25.—Illustrated key to the cestode parasites of man. By Ch. Wardell Stiles.

No. 26.—On the stability of the oxidases and their conduct toward various reagents. The conduct of phenolphthalein in the animal organism. A test for saccharin, and a simple method of distinguishing between cumarin and vanillin. The toxicity of ozone and other oxidizing agents to lipase. The influence of chemical constitution on the lipolytic hydrolysis of etheral salts. By J. H. Kastle.

*No. 27.—The limitations of formaldehyde gas as a disinfectant with special reference to car sanitation. By Thomas B. McClintic.

*No. 28.—A statistical study of the prevalence of intestinal worms in man. By Ch. Wardell Stiles and Philip E. Garrison.

*No. 29.—A study of the cause of sudden death following the injection of horse serum. By M. J. Rosenau and John F. Anderson.

No. 30.—I. Maternal transmission of immunity to diphtheria toxine. II. Maternal transmission of immunity to diphtheria toxine and hypersusceptibility to horse serum in the same animal. By John F. Anderson.

No. 31.—Variations in the peroxidase activity of the blood in health and disease. By Joseph H. Kastle and Harold L. Amoss.

No. 32.—A stomach lesion in guinea pigs caused by diphtheria toxine and its bearing upon experimental gastric ulcer. By M. J. Rosenau and John F. Anderson.

No. 33.—Studies in experimental alcoholism. By Reid Hunt.

No. 34.—I. *Agamofilaria georgiana* n. sp., an apparently new roundworm parasite from the ankle of a negress. II. The zoological characters of the roundworm genus *Filaria* Mueller, 1787. III. Three new American cases of infection of man with horse-hair worms (species *Paragordius varius*), with summary of all cases reported to date. By Ch. Wardell Stiles.

*No. 35.—Report on the origin and prevalence of typhoid fever in the District of Columbia. By M. J. Rosenau, L. L. Lumsden, and Joseph H. Kastle. (Including articles contributed by Ch. Wardell Stiles, Joseph Goldberger, and A. M. Stimson.)

No. 36.—Further studies upon hypersusceptibility and immunity. By M. J. Rosenau and John F. Anderson.

No. 37.—Index-catalogue of medical and veterinary zoology. Subjects: Trematoda and trematode diseases. By Ch. Wardell Stiles and Albert Hassall.

No. 38.—The influence of antitoxin upon post-diphtheritic paralysis. By M. J. Rosenau and John F. Anderson.

No. 39.—The antiseptic and germicidal properties of solutions of formaldehyde and their action upon toxines. By John F. Anderson.

No. 40.—1. The occurrence of a proliferating cestode larva (*Sparganum proliferum*) in man in Florida, by Ch. Wardell Stiles. 2. A reexamination of the type specimen of *Filaria restiformis* Leidy, 1880=*Agamomermis restiformis*, by Ch. Wardell Stiles. 3. Observations on two new parasitic trematode worms: *Homalogaster philippinensis*

n. sp., *Agamodistomum nanus* n. sp., by Ch. Wardell Stiles and Joseph Goldberger. 4. A reexamination of the original specimen of *Tænia saginata abietina* (Weinland, 1858), by Ch. Wardell Stiles and Joseph Goldberger.

*No. 41.—Milk and its relation to the public health. By various authors.

No. 42.—The thermal death points of pathogenic micro-organisms in milk. By M. J. Rosenau.

No. 43.—The standardization of tetanus antitoxin (an American unit established under authority of the act of July 1, 1902). By M. J. Rosenau and John F. Anderson.

No. 44.—Report No. 2 on the origin and prevalence of typhoid fever in the District of Columbia, 1907. By M. J. Rosenau, L. L. Lumsden, and Joseph H. Kastle.

No. 45.—Further studies upon anaphylaxis. By M. J. Rosenau and John F. Anderson.

No. 46.—*Hepatozoon perniciosum* (n. g., n. sp.); a hæmogregarine pathogenic for white rats; with a description of the sexual cycle in the intermediate host, a mite (*lelaps echidninus*). By W. W. Miller.

No. 47.—Studies on Thyroid: I. The relation of iodine to the physiological activity of thyroid preparations. By Reid Hunt and Atherton Seidell.

In citing these bulletins, beginning with No. 8, bibliographers and authors are requested to adopt the following abbreviations: Bull. No. ——, Hyg. Lab., U. S. Pub. Health & Mar. Hosp. Serv., Wash., pp. ——.

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TREASURY DEPARTMENT
Public Health and Marine-Hospital Service of the United States

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HYGIENIC LABORATORY.—BULLETIN No. 48
DECEMBER, 1908

THE PHYSIOLOGICAL STANDARDIZATION OF DIGITALIS

By

CHARLES WALLIS EDMUNDS
and
WORTH HALE

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THE PHYSIOLOGICAL STANDARDIZATION OF DIGITALIS.[a]

By Charles Wallis Edmunds,
Assistant Pharmacologist, Hygienic Laboratory, U. S. Public Health and Marine-Hospital Service,

and

Worth Hale,
Assistant Pharmacologist, Hygienic Laboratory, U. S. Public Health and Marine-Hospital Service.

One of the directions along which scientific medicine has advanced during the past few years is in the greater accuracy with which medicines are administered. A very essential factor in this tendency is that the drugs themselves shall be of a uniform strength, and to insure this the Pharmacopœia of the United States, in its last revision, provided a number of standards to which official preparations must conform. Especially is this true in the case of a number of drugs, such as opium, belladonna, nux vomica, etc., in which the active constituents are capable of isolation in a pure form. With a considerable number of others, however, this was not found to be possible for the reason that the active principles are either not known or are not capable of being isolated in pure state by any known chemical method. In this class occur such drugs in common use as digitalis and the other members of that series, ergot and cannabis indica.

By far the most important member of this group is digitalis itself, on account of its widespread use in cases of cardiac disease. At the present time it is impossible to secure a standardized preparation of the drug by any known chemical means[b] on account of the fact that the activity of the drug depends upon no single active principle, but upon several whose chemistry is not completely known and for the isolation of which there does not, at the present time, exist any satisfactory chemical method.

Our knowledge of the chemistry of the digitalis leaves we owe mainly to Schmiedeberg and Kiliani. The most important constituent is digitoxin, a glucoside, which is insoluble in water but soluble

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[a] Submitted for publication December 4, 1908.

[b] Some firms put out preparations standardized so as to contain a certain percentage of digitoxin, but, as will be pointed out, this need not indicate a uniform physiological activity.

in alcohol and chloroform. In watery solutions of the drug it is probably made soluble by the presence of other constituents of the plant, most likely the saponin-like body, digitonin. The digitoxin is readily broken up into digitoxigenin and the sugar, digitoxose. Schmiedeberg obtained toxiresin as a decomposition product, a body which is related in action to the picrotoxin group.

A second glucoside is digitalinum verum or digitalin, which is soluble in alcohol but insoluble in chloroform or water. This breaks up into an inactive body digitaligenin and two sugars. The digitalinum verum is found mainly in the seeds.

Digitalein[a] resembles digitalin very closely, differing from it, however, in being soluble in water.

Digitophyllin, another glucoside, which has been isolated, resembles digitoxin.

While digitoxin is the most important constituent of the preparations of digitalis it does not necessarily follow that the activity of the drug runs parallel with the digitoxin content. At least this is true as far as we can judge by the method which is available for the isolation of this body, viz, the Keller method or the Keller method as modified by Fromme. Keller's method[b] for the isolation of digitoxin consists in the extraction of a certain amount of carefully dried and powdered leaves with dilute alcohol. The percolate is evaporated to drive off the alcohol and the residue is taken up with water and lead acetate added. A voluminous precipitate results, but only the first portion of the filtrate obtained is saved to avoid the uncertainty of attempting to wash the precipitate free from digitoxin. Sodium sulphate is added to the filtrate to precipitate any excess of lead and after standing four to five hours the clear liquid is decanted, made alkaline with ammonia, and shaken out with chloroform. The chloroformic solution is evaporated and a yellow resinous mass, the digitoxin, is left behind. This may be purified by taking it up with a small amount of chloroform and reprecipitating with a mixture of sulphuric and petroleum ether.

Several workers have tried to find a relationship between the digitoxin content and the activity of the preparations, as determined by biological methods, but the results, in almost every case, have proved a failure, as will be seen by a study of the following tables, which we have brought together for the sake of comparison.

Ziegenbein[c] conducted such a series of comparative experiments, estimating the toxic dose upon frogs, employing leaves obtained from various localities.

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[a] According to Kiliani, München. med. Wchnschr., 1907, LIV, 886, the widely advertised proprietary preparation digalen consists of a solution of impure digitalein.

[b] Keller, Ber. d. deutsch. pharm. Geselssch., Berl., 1897, VII, 125.

[c] Ziegenbein, Arch. d. Pharm., 1902, CCXL, 454.

No.	Specimen.	Digitoxin content.	Toxic dose for 100 gram frog.
		Per cent.	*Gram.*
1	Leaves from North Harz, 1901	0.33	0.03
2	Leaves from North Harz, 1901 (selected)	.163	.03
3	Leaves from South Harz, 1901	.14	.04
4	Leaves from South Harz, 1901 (selected)	.185	.03
5	Leaves from North Harz, 1901	.125	.03
7	Leaves from Thuringia	.115	.05
10	Leaves, old, 1900	.226	.06
11	Leaves, Harz (finely powdered)	.235	0.04-.05
12	Leaves, Harz (finely powdered)	.18	.05

Famulener and Lyons[a] carried out a similar research with the following results:

Specimen fluid extract of digitalis.	Purified digitoxin content.	Toxic dose for 40 gram frog.
	Per cent.	*Gram.*
A	0.196	0.026
B	.258	.029
C	.182	.038

Barger and Shaw[b] found that the ratio of toxicity of the tincture of digitalis to the toxicity of the digitoxin contained in the specimen varied in the series of nine tinctures from 3.5 : 1 to 6.9 : 1, or, in other words, tinctures from 3.5 to 6.9 times as toxic as the digitoxin which was obtained by chemical assay. Focke[c] and Fromme carried out a similar set of comparative experiments and concluded that the digitoxin content and the physiological activity as determined by experiments on the frog did not run parallel. Reed[d] and Vanderkleed, using guinea pigs, claimed to have found a certain parallelism, but a study of their results shows that the parallelism is by no means without exceptions:

No.	Preparation.	Chemical assay. Gram digitoxin in 100 cubic centimeters.	Physiological assay. M. L. D.[e] for guinea pig of 240 grams weight.
		Grams.	*c. c.*
1	United States Pharmacopœia tincture	0.0377	0.6
2	.....do	.023	1.0-1.25
3	.....do	.0277	.75
4	.....do	.0254	1.0
5	Fat free tincture	.027	1.0-1.25
6	Fluid extract	.264	0.1
7	.....do	.2405	.09 .1
8	.....do	.234	.08
9	Powdered extract	[f]1.061	[g].019-0.025

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[a] Famulener and Lyons, Proc. Am. Pharm. Ass., Phila., 1902, L, 415.

[b] Barger and Shaw, Chem. and Physiol. Assay of Digitalis Tinctures, Handbook of Pharmacy, 1904, 541; Pharm. J. and Tr., 1904, LXXIII, 249.

[c] Focke, Deutsch aerzt. Zeit., 1904, VI, 292.

[d] Reed and Vanderkleed, Am. J. Pharm., Phila., 1908, LXXX, 110.

[e] M. L. D.=minimum lethal dose.

[f] Per cent.

[g] Gram.

These figures certainly show a closer relationship than has been obtained by the previous workers.

Since we have then no satisfactory chemical method of estimating the activity of digitalis preparations, attention has been directed toward the development of assay by physiological methods. Several have been employed in the past ten years and the results obtained by their aid have served to emphasize the necessity of some way of standardizing these drugs. In both this country and in Europe many writers have demonstrated by these physiological tests the great variability of strength which exists not only in the galenical preparations of the drug but also in the so-called pure preparations themselves.

Among these investigators may be mentioned Bennefeld, who in 1881 showed that for rabbits the lethal doses of eight digitalis tinctures varied about fourfold.

Bührer (1900) demonstrated on frogs that some of the fluid extracts of digitalis were four times as strong as others.

In 1902 Fränkel showed that six infusions of digitalis varied from 100 to 275 per cent, and six tinctures from 100 to 400 per cent.

Siebert (1903) found in fifty experiments on digitalis leaves that the fatal doses per 100 gram frogs varied from 0.03 to 0.075 gram.

Edmunds in 1907 showed that seventeen tinctures of digitalis purchased in the open market varied in strength as 1 to 4, while Gottlieb (1908) found the same ratio to exist in Heidelberg. Reed and Vanderkleed (1908) found the toxic dose for guinea pigs of 240 grams weight varied in four tinctures from 0.6 to 1.25 c. c.

These variations in strength depend upon several factors, among which may be mentioned the source of the plant; for example, Ott[a] has pointed out that Bohemian leaves are more toxic than others. At the present time the English leaves are considered the best. The plants growing wild are preferred to the cultivated variety, and those growing in sunny places to those in the shade, and those grown in dry seasons to those gathered in rainy. However, the most important factor in determining the activity of the leaves is the manner of drying and the mode of preservation, the influence of which has been especially studied by Focke (p. 24).

The experiments of Bennefeld and others to determine the relative activities of different members of the group were carried out along essentially the same lines and embody the same principles as are employed to-day in the biological standardization of these drugs. It was but a step from one to the other; the first writers determined the relative activity, while the later writers merely corrected these variations by such means as concentration or dilution of the preparations. To get then any comprehensive idea of the development of modern physiological standardization it is necessary to go further back than the first paper strictly devoted to the standardization problem and examine the earlier methods in vogue. We have therefore given short abstracts from earlier writings of such articles as bear upon the subjects before considering it in the light of more recent works, not only digitalis being discussed, but some other drugs belonging to the same group, such as strophanthus, as the same methods apply to all the members of the series.

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[a] Ott, Verhandl. d. Cong. f. innere Med., Wiesb., 1901, 89.

In 1865 Fagge and Stevenson, in an address before the Royal Society, claimed that physiological tests would be of great medico-legal importance.[a] They had studied digitalin especially and said for this purpose frogs were better adapted than the higher animals, as the various organs could be examined, the animals exhibited no fear, and the drugs acted quickly upon them.

In their paper published in 1866 they made a report upon the relative activity of several members of the digitalis group, among them being "antiar," helleborus viridis, squills, digitalin, and the infusion of digitalis. The method employed was to weigh the frog, attach it to a piece of cork, and expose the heart, avoiding hemorrhage as far as possible. It was then injected beneath the skin of the thighs and flanks and the time noted at which systolic standstill of the ventricle occurred. The length of time between the injection of the drug and the stoppage of the heart determined the relative toxicity of the different drugs. Fagge and Stevenson considered that the season of the year did not alter the reaction of the frogs to the drugs.

Ten years later Koppe[b] published the results of his investigations upon the relative toxicity of the active constituents of digitalis, digitoxin, digitalin, and digitalein. His experiments were carried out upon frogs, cats, dogs, and rabbits. In his work on frogs he employed both Rana temporaria and R. esculenta and showed how much more susceptible the former were than the latter. His method was to expose the heart in the usual manner and inject the drugs, noting the time when systolic standstill of the heart appeared.

The dogs were either injected subcutaneously or the drug was administered by the mouth, changes in the rate of the heart and the strength of the beat being noticed, as well as the time when nausea and vomiting occurred. To cats the drug was administered both subcutaneously and by the mouth and the amount necessary to produce vomiting noted. Large doses produced alterations in the pulse rate. The drugs were given subcutaneously to rabbits and produced in them as the most prominent symptom a more or less complete paralysis associated with irregularity and slowing of the heart rate.

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[a] C. Hilton Fagge and Thomas Stevenson, Application of physiological tests for certain organic poisons, and especially digitaline. Reprinted from Guy's Hosp. Reports, 1866.

[b] Koppe, Arch. f. exper. Path. u. Pharmakol., Leipz., 1875, III, 274.

Fr. Bennefeld[a] in 1881 undertook an investigation of the relative activity of tinctures of digitalis obtained from different parts of Germany. He first tried to compare them by means of their specific gravities and the amount of the dried residue, but his results, confirming those of Schneider,[b] showed that such examinations were worthless. He then adopted the physiological method, employing rabbits as experimental animals.

The tinctures were prepared for examination by evaporating 25 c. c. and drying the resulting residue to constant weight over sulphuric acid. The residue was digested on a water bath for fifteen minutes at a moderate temperature with 15 c. c. of distilled water, was then filtered as quickly as possible and brought to a volume of 20 c. c. Before injection it was heated to body temperature.

The rabbits used were of the same age, weight, and resistance as far as possible. After inserting a cannula into a jugular vein, the modified tincture was injected at regular intervals in 0.5 c. c. doses until the animal died or the 20 c. c. was exhausted.

His results showed that the minimum lethal dose for the eight tinctures varied from 3.36 to 15.97 c. c. of the residue solution per kilogram, body weight, while the time of death varied from eleven to fifty-four minutes.

Frankel[c] adopted still another method to determine the comparative activity of different preparations of digitalis. Dogs weighing between 10 and 18 kilograms were curarized and the drug injected subcutaneously in doses not exceeding 10 c. c. His standard for comparison consisted in the amount which was necessary to produce the digitalis effect, viz, a decrease in the rate of the heart with an increase in the volume of the single beats. He also registered the blood pressure.

He found marked differences between the various preparations in the amount necessary to bring about the desired effects; which he thought might be partly due to individual variations in the dogs and also possibly to the curara. The tincture was found to be very weak, while the acetum was the stronger preparation, but the active amounts of both acetum and infusion were quite variable.

Laborde and Duquesnel[d] examined two samples of digitalien (Nativelle) as to their chemical purity and physiological activity, using for the latter purpose frogs and guinea pigs.

The hearts of the frogs were exposed and the drugs injected in 5-milligram doses, the effects being noted. With one preparation the heart stopped in systole in two minutes, while with the second sample it continued to beat for hours. The difference in physiological activity was also shown by means of graphic records.

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[a] Fr. Bennefeld, Über Digitalis-Tincturen, Comparativ-experimentelle Untersuchungen, Inaug. Dissert., Göttingen, 1881.

[b] von Schneider, Arch. d. Pharm., 1879, CCXV, 412.

[c] Frankel, Charité-Ann., Berl., 1881, VI, 207.

[d] Laborde and Duquesnel, Comp. rend. hebd. des Séances et Mém. de la Soc. de Biol., 1884, XXXVI, 93.

The results obtained on the frogs were confirmed by the injection of 5 milligrams of each sample into two guinea pigs, the length of time till death occurred serving as a means of comparison.

Similar methods of comparison were also employed by Laborde[a] in his study of the purity and activity of the digitalines in use at the hospitals of Paris.

Gley[b] compared the toxicity of ouabaine and strophanthin by means of experiments upon several varieties of animals. Upon frogs he showed that with equal doses of the two drugs ouabaine would stop the heart in half the time that strophanthin would. He then compared the toxic dose of the two drugs upon guinea pigs, dogs, and rabbits; in the latter case injecting the drugs into the vein of the ear, in the other cases using subcutaneous injection.

Reusing[c] carried out a series of experiments comparing the actions of strophanthin and digitalis upon the frog's heart, employing two methods in his work. In the first he studied the effect directly upon the exposed heart, the pericardium not being opened. In the second method the Ludwig-Coat's heart apparatus was employed, the perfusion fluid to which the drugs were added consisting of two parts of 0.65 per cent NaCl solution with 1 part of defibrinated hog blood.

Bardet[d] undertook in the same year an examination of the relative activity of the various active principles of digitalis and employed the simplest method which is in use to-day to standardize these drugs, viz, that of determining the lethal dose for animals, using frogs and rabbits.

An examination of a number of the so-called digitalines of commerce was made by Fouquet,[e] who ascertained the toxic dose of the different samples when they were injected hypodermically into frogs, rabbits, and dogs. In this way he studied the relative activity of crystallized and amorphous digitalin, digitoxin, and digitalein.

In 1893 Prevost[f] examined a number of the preparations which had been included in the Swiss Pharmacopœia III, among them being some members of the digitalis group. His experiments were carried out upon frogs (mainly R. temporaria), upon which he sought the minimal dose of the drug which would produce definite stoppage of the heart and death of the animal. He considered the frog as the most suitable animal upon which to test the relative activity of the members of this group as mammals vary considerably in their susceptibility to them. In his earlier experiments he did not weigh the animals, but later adopted this precaution, regarding the weight as being a factor of some importance, but at the same time not considering that the action of the drug upon different frogs is in any absolute relation to their weights. The frogs he employed were an average size, being between 25 and 30 grams. He confirmed some of the results obtained on frogs by experiments upon guinea pigs, the comparative results being as follows:

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[a] Laborde, Comp. rend. hebd. d. Sèan. et Mém. de la Soc. de Biol., 1884, XXXVI, 599.

[b] Gley, Comp. rend. des Sèances de l'Acad. de Sci., 1888, CVII, 348.

[c] Reusing, Über die Einwirkung des Strophanthin auf das Froschherz. Inaug. Dissert, Würzburg, 1889.

[d] Bardet, Bull. et Mém. Soc. de thérap., Par., 1889, 2d ser., XVI, 253.

[e] Fouquet, Bull. gén. de thérap., 1892, CXXII, 71.

[f] Prevost, Rev. Méd. de la Suisse Rom., Genève., 1893, XIII, 505.

Animal used.	Toxic dose.		
	Fluid extract convallaria.	Fluid extract digitalis.	
		No. 26.	No. 6.
	c. c.	c. c.	c. c.
R. temporaria, average size	0.003	0.02	0.04
Guinea pig, per 100 grams	.006	.01	.02

It will be seen that the two digitalis preparations show exactly the same ratio on both animals.

In 1895 Prevost [a] repeated his experiments upon these preparations to see if they had deteriorated in the intervening time. In this case he used R. esculenta, realizing, however, that they did not offer the same susceptibility as R. temporaria.

Mlle. M. Piotrowska [b] studied the comparative toxicity of a number of the members of this group and the effect upon their toxicity of the mode of administration, whether subcutaneously or by the mouth. For this purpose she used frogs (R. temporaria) almost exclusively, determining the toxic dose and comparing them on a basis of 100 grams body weight. Some of her results she confirmed by determining the toxic dose for cats and rabbits, comparing them on the basis of kilograms of body weight.

The examination of the plant dialysates was made the subject of investigations by several workers, one of the earliest being Jacquet.[c] These dialysates were prepared by Golaz of Vevey, Switzerland, from fresh plants and made of such strength that 1 part of dialysate corresponded to 1 part of leaves. By using great care in the collection of the leaves, gathering them in the same region during sunny weather, using them fresh, and making the product up to a uniform volume, it was believed that a definite dosage of digitalis was attained, as so many of the disturbing factors had been eliminated. It was naturally impossible to make allowances for the yearly variation which was known to take place, and therefore the argument for a uniform product based upon these facts alone falls to the ground. Jacquet, who pointed out in his paper these advantages of the dialysates, made a number of physiological tests on the dialysates of digitalis and Adonis vernalis. He employed frogs (R. esculeuta and temporaria) with exposed hearts, injecting the drug into the lymph sacs and noting the length of time before death. He also studied their action upon the blood pressure and heart rate in rabbits and noted the toxic dose.

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[a] Prevost, Rev. méd. de la Suisse Rom., Genève., 1895, XV, 453.

[b] M. Piotrowska, Inaug. Dissert., Geneva, 1896.

[c] A. Jacquet, Cor.-Bl. f. schweizer Aerzte, 1897, XXVII, 326.

By a comparison of the results obtained in this way with those he obtained under similar conditions using digitoxin and digitalium verum he estimated the dose of the dialysates which it would be proper to employ in man, it being possible to do this as the therapeutic dose of the active principles for man was already known.

It appears that Jacquet approached more nearly to physiological standardization than any of his predecessors, as he points out the advantage of the dialysates in affording an exact dosage, but it was not until his second paper,[a] which appeared in December, 1898, that he seems really to have adopted physiological standardization. As stated above, he believed that a uniform preparation had been obtained by eliminating so many factors which go to make the great variations which are found in the ordinary galenical preparations; but as he stated then, he was unable to avoid the yearly variation in plants. This was evidently quite a large factor, as, examining the 1896 specimen, he was able to isolate 0.16 per cent of the active constituent, while in the 1897 preparation there was only 0.096 per cent. This variation he ascribed to the fact that the weather was unsettled at the time of gathering the leaves, and on that account the plants contained an unusually large amount of water. He therefore compared the two dialysates by physiological methods and found the same relative differences, for while ten drops of the 1896 specimen produced systolic standstill in frogs, it took twenty drops of the 1897 preparation. Likewise, it took double the amount of the 1897 preparation to kill rabbits.

Recognizing at this time the yearly variation, which is apparently of a good deal of importance, he suggested that either a yearly change of dose would be necessary or an adjustment of the solution by evaporation or dilution as might be necessary, so as to give a uniform product. The latter course he thought preferable. This paper, in which he really adopted the physiological method of standardization, was, however, preceded by one by Houghton which appeared in America in October, 1898.

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[a] A. Jacquet, Cor.-Bl. f. schweizer Aerzte, 1898, XXVIII, 745.

No reference is directly made in Jacquet's paper as to the effect of the weight of the frogs upon the results, except, in the protocols, the size is noted as "large" or "medium."

In his experiments upon rabbits he used two methods. Upon most he measured the effect upon the blood pressure and heart rate when the drug was injected into the jugular vein and calculated also the toxic dose per kilogram body weight. In other cases he injected the drug subcutaneously.

It appears strange that, although so much work had been done on the comparison of the relative strengths of the different members of the digitalis group, no practical application was made of the methods developed until more than thirty years after Fagge and Stevenson published their results. As pointed out, it was not until 1898 that the application of these physiological tests was advised for the production of uniform pharmaceutical preparations. In that year Houghton[a] published his method for the physiological assay of strophanthus, basing it upon the fact that the killing power of the cardiac drugs for frogs of definite size and species, kept under proper conditions, is constant per gram of body weight. He had tried rabbits, guinea pigs, and rats, as well as frogs, but came back to the latter as being most satisfactory as he found too great variation in the blood pressure of dogs and rabbits under the action of digitalis to allow of this method being of use. Any species of frogs may be employed, provided both the standard solution and the unknown preparation are tested upon the same species, for, while animals of different species vary in their reaction, those belonging to the same are much alike. Also frogs of any weight may be used, providing the proper allowance is made for the variation, but in testing a preparation, it is best to use frogs of nearly uniform size, varying from one another not more than 3 grams. The frogs to be employed should be freshly caught and carefully handled, being kept in wet moss until they can be placed in suitable pools at the laboratory.

The dose of the drug to be tested is calculated according to the weights of the frogs to be employed and made up with salt solution so that it will measure about one-half cubic centimeter, and is then introduced into the abdominal lymph sac by means of a pipette. The frogs are placed in separate jars and allowed to remain for twelve hours, at the expiration of which time they are examined and note made as to the dead and living animals. By a preliminary test in which the limits of dosage are quite wide some idea is formed as to the strength of the preparation and a second series of frogs is injected, still further narrowing the dose. When by this second group the probable toxic dose is obtained, a new series of five frogs is injected, and if the dose is correct at least three frogs out of the five should be killed. By a simple calculation the relative strengths of the unknown and the standard preparation can then be obtained.

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[a] Houghton, J. Am. M. Ass., Chicago, 1898, XXXI, 959.

An important contribution to the subject of biological assay was furnished by Carl Bührer,[a] who investigated the activity of some of the toxic fluid extracts, among them being digitalis and convallaria. He selected frogs and rabbits as experimental subjects. The frogs, either R. esculenta or temporaria, were chosen with great care and obtained fresh every two weeks, but he used only one species for one preparation and for digitalis only R. temporaria. He seems to be the first to note any effect of sex upon the results and says he finally employed females exclusively. The effect of season upon the reaction of the frogs was corrected by repeating his experiments at various times of the year. Bührer seems not to have considered the size of the animal as being important, for while he weighed the frogs before the drug was injected, the weight was not used in calculating the dose or judging the results. The method he used was as follows: The frog was stretched out on its back and then, with as little hemorrhage and other injury as possible, its heart was exposed. The rate was counted at once and again after five minutes when the drug was injected into the thigh lymph sac and the animal put into a moist chamber and observed at intervals. The dose of the drug to be tested was prepared by diluting the fluid extract with different amounts of salt solution and injecting one-half cubic centimeter. The end reaction or standard consisted in finding the smallest amount of drug which would cause systolic stoppage of the heart within twenty-four hours. The comparative strengths of the different specimens were then reckoned as so many milligrams, according to the dilution of the drug, without paying any attention to the weight of the animal. By this method he found the specimens of 1897 varied in the ratio of 1 to 4, while the 1898 fluid extracts were weaker, but more uniform in strength. He though it was possible that this apparent weakness was due to a difference in the reaction of the frogs, as the 1898 specimens were examined one and one-half months after those of 1897, and he had found that even in from two to four weeks the animals might alter very considerably in their reaction to one and the same drug. This variation he thought was due to their winter fast, but in this case his supposition was proved incorrect by subsequent examination made in December, 1899.

Bührer further continued his experiments, employing rabbits which were arranged for blood pressure estimations. The diluted drug was injected intravenously at intervals of from five to ten minutes, until the lethal dose was reached. By carrying out several experiments an average lethal dose was obtained, which was based upon the toxic amount per kilogram body weight.

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[a] Bührer, Untersuchungen über die Wirksamkeit einiger toxischer Fluid-Extrackte, Inaug. Dissert., Basel, 1900.

The variations obtained on rabbits did not appear as great as with the frogs, being only 200 instead of 400 per cent. He tabulates his results as follows:

Fluid extract digitalis.	1897.		1898.	
	Frogs.	Rabbits.	Frogs.	Rabbits.
	Mg.	*Mg.*	*Mg.*	*Mg.*
A	17	400	33	590
C	25	630	25	780
D	33	770	25	740
B	67	890		

The relation between the different preparations is not the same with the frogs as it is with the rabbits, and Bührer had expected this discrepancy, as he said the two animals are so entirely different. However, fluid extract A is shown to be the strongest by both methods

Fränkel[a] assayed Merck's digitoxin and later took up different infusions of digitalis which he obtained in and around Heidelberg. His method was to ascertain the amount of drug which would produce systolic standstill of the frog's heart in about an hour's time. If the heart stopped in a shorter time than fifty minutes the dose was lessened, while if it had not ceased to beat in one hour and ten minutes the dose was increased. The frogs (species not mentioned) were injected in a lymph sac, the amount of fluid being usually 1 c. c. except in some cases 2 or even 3 c. c. were employed. The animals were weighed and the toxic dose per 100 grams body weight calculated by a simple ratio.

His results showed that six infusions varied in toxic dose from 2.5 to 6.9 c. c., a difference of 100 to 275 per cent. Six tinctures examined varied from 0.6 to 2.5 c. c., 100 to 400 per cent, and seven strophanthus tinctures varied from 0.015 to 1.0 c. c. Fränkel thinks we are justified in transferring these results to the higher animals as Bührer was able to show a parallelism between the action on rabbits and on frogs, while he himself with four digitalis preparations showed the same ratio to exist on cats as on frogs. (Bührer's table, as he himself pointed out, shows a certain parallelism, but by no means an exact one.)

In 1902 Famulener and Lyons[b] undertook a study of the relative strength of various preparations of digitalis and related drugs. They employed frogs (species not mentioned), choosing those of about 40 grams weight and injecting the preparations to be tested into the ventral lymph sac by means of finely pointed pipettes. At the end of an hour the heart of the animal was exposed and examined. If the heart was found completely paralyzed the dose was too large; if pulsation still continued the amount was too small. The correct "end reaction" adopted by the writers was "paralysis" of the apex of the ventricle, but at the base of the ventricle there should occasionally be a faint wave of contraction while the auricle, though much distended, would continue to pulsate regularly. When the correct dose was found the amount necessary to kill a frog of standard weight (40 grams) was calculated by a simple proportion.

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[a] Fränkel, Therap. d. Gegenw., Berlin u. Wien, 1902, XLIII, 106.

[b] Famulener and Lyons, Proc. Am. Pharm. Ass., Phila., 1902, L, 415.

Famulener and Lyons found the reaction of the frogs very uniform, for not more than one or two out of a dozen healthy frogs showed a variation in susceptibility of as much as 10 per cent. To avoid this source of error, when they had determined the minimum dose in the manner indicated, they took a further series of three frogs, injecting into one the minimum dose, into a second a dose 10 per cent above the minimum, and into the third a dose 10 per cent below it. If any of this latter series showed irregularities further corrections were made. More uniform results were obtained by this method than could be obtained in the assay of such drugs as opium and nux vomica by chemical means. The authors give a table showing the results of their examination into the activity of the pure principles of digitalis and related drugs as well as of the galenical preparations. A very important contribution in their paper is a comparative study of the digitoxin content of three preparations of digitalis fluid extract with their activity as determined by physiological methods. This was referred to earlier in our article (p. 9).

Wolff[a] made a short report upon the biological dosage of digitalis preparations commenting upon the findings of Fränkel and quoting Professors Kobert and Gottlieb upon the necessity for a government station for the standardization of digitalis preparations. He then describes the method adopted by Brunnengräber, of Rostock, to bring into the market uniform preparations of these drugs. The leaves are collected on sunny days from wild plants before they begin to flower, carefully sorted to free them from any foreign leaves, and are then dried quickly in a vacuum at a definite temperature. In this way the fermentation which the leaves usually undergo during slow drying is avoided. Tinctures prepared from leaves, dried in this way, were examined by Professor Kobert, who reported them free from harmful split products and high in content of active principles. Strophanthus tinctures were also prepared from the fresh green seeds of S. Kombé which had not been freed from fat and these too were very active. An infusion made from 5 milligrams of digitalis leaves produced systolic standstill of the frog's heart as did also 0.02 c. c. of the tincture of strophanthus.

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[a] Wolff, Therap. d. Gegenw., Berlin u. Wien, 1902, XLIII, 423.

Ziegenbein[a] during 1901 and 1902 examined a number of specimens of digitalis leaves obtained from different localities estimating the digitoxin content and conducting comparative experiments by physiological methods. His results as given in the table on page 9 show that no definite parallelism between the two can be found. The toxicity of the digitoxin, which he was able to isolate by the Keller or Keller-Fromme method, only accounted for about one-third of the activity of the leaves from which it was obtained. He examined the toxicity of the extract which was left after shaking it out with chloroform to remove the digitoxin, and found that some principles which were insoluble in chloroform still remained and accounted for another one-third of the activity of the leaves, but the remaining one-third could not be accounted for. He carried out some experiments to determine the effect of the fineness of the drug upon the toxicity of the infusion which when powdered gave a higher value than did infusions made from leaves which were simply cut up. The toxic dose for the former was found to be 0.048 gram, while the latter required 0.064 gram to produce the same effect, and when finely powdered the leaves were most potent requiring only 0.03 gram, the difference probably being due to the greater surface of the drug exposed to the solvent. The method he employed for the physiological estimations was that originated by Hans and Arthur Meyer. In this method, freshly caught male frogs (R. temporaria) were secured upon a board and the heart exposed in the usual manner. Definite amounts of the drug to be tested were then injected into the thigh lymph sac and the smallest amount was sought which would produce systolic standstill within two hours. The minimum lethal doses thus found were calculated upon the basis of 100 grams frog weight in order to allow of comparisons. Frogs weighing about 25 grams were chosen for the experiments, but Ziegenbein did not think that a few grams difference in weight influences the results to any appreciable extent. He was also of the opinion that the season of the year makes very little difference in the susceptibility of frogs.

In the following year Moschkowitsch[b] published a critical study of the method of standardizing digitalis preparations by means of their action on frogs. His experiments were carried out on R. temporaria, which were fastened to a board and the sternum removed to permit of free observation of the heart, the pericardium being left intact to prevent drying. The drug to be tested was introduced into a lymph sac and its activity was measured by the time when it first produced an effect on the heart and by the time necessary to cause systolic stoppage.

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[a] Ziegenbein, Arch. d. Pharmacie, 1902, CCXL, 454. Ber. d. Deut. Pharm. Gesells., 1902, XII, 335.

[b] H. F. Moschkowitsch, Arch. d. Pharm., 1903, CCXLI, 358.

The author studied pure principles and also galenical preparations, and the most important fact which he brought out and especially emphasized in his work was the great variability in the reaction of frogs. The time of the first appearance of the action varied between two and six minutes, but a still greater variability was shown in the time of the appearance of systolic standstill. He said he was greatly discouraged by the great capriciousness in the reaction of the frogs, and the hope of an exact estimation by physiological methods received a mighty blow.

While in the estimation of his dosage he did not usually take into consideration the weight of the animal, yet in discussing his results he had several opportunities to compare the action of the drug upon animals of the same size. In some of his tables this factor seems to be important, while in others it would appear that it could be ignored; the hearts of frogs of the same size given the same dose stopping in widely different times, while those of different sizes stopped after like intervals when given the same sized dose. His conclusion upon this phase of the question was that it was best to take into consideration the weights of the frogs in making an assay. Upon the important question as to the effect of the season upon the susceptibility of frogs he found that summer (July) frogs possessed far greater resistance than winter frogs.

He points out that his results do not confirm those of the earlier workers, but he thinks the individuality of the animal is an important factor. According to this author, unless animals of about the same weight and of the same species can be obtained from a certain section of the country at the same time of year greater reliance can be placed upon chemical analysis. At any rate, he concluded that it was only in a very general way that we could tell from physiological experiments as to whether a preparation was good or bad, and while the method was not necessarily useless it was defective.

In the same year Brondgrest[a] also conducted a series of experiments to compare the action of the dialysate of digitalis grandiflora with digitalinum purum and the infusion of digitalis purpura. He employed for this purpose the frog's heart in situ, using the suspension method combined with the Williams perfusion apparatus. He concluded that the dialysate of digitalis grandiflora acts the same as D. purpuræ and as digitalinum.

Among the numerous writers upon the subject of standardization no one has contributed more than Focke,[b] who published several articles between 1902 and 1906. His method has undergone one or two important modifications since it was originally published, but otherwise it is essentially the same. He employs frogs (Rana temporaria) which are collected not earlier than the end of June. They are kept in a cellar in a box the bottom of which is covered with water, the latter being changed at least daily. The frogs should not be used before the third day of captivity and should be brought to the examining room six hours before they are needed, being placed in jars at a temperature not over 17° C. The examinations should be carried out in July, August, and September, during which months the susceptibility of the frogs does not differ greatly, and also during this time no distinction need be made with reference to sex. Animals weighing between 25 and 30 grams are selected and fastened to a board in the usual manner, and the heart is exposed without allowing bleeding or other injury (pithing is not permitted at this time). After opening the pericardium a slight pressure upon the abdomen will cause the heart to protrude from the opening, where it will remain in plain view. A measured quantity of the infusion to be tested is now injected into the two leg lymph sacs, about 0.3 c. c. into each or 0.6 c. c. in all. Both lymph sacs are used so that the distended skin will not force the drug out of the opening by pressing upon a large bulk of fluid. Systolic standstill of the heart is now watched for and it should appear in ten or fifteen minutes. When this is observed and the time noted, the frog is pithed and weighed.

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[a] Brondgrest, Zentrbl. f. innere Mid., Leipz., 1903, No. 24, 906.

[b] Focke, Therap. d. Gegenw., Berlin u. Wien, 1902, XLV, 44; Arch. f. Pharm., 1903, CCXLI, 128, 669; Deutsche Aerzte Ztg., Berlin, 1904, VI, 272, 292; Therap. d. Gegenw., Berlin u. Wien, 1904, XLV, 250; Berlin klin. Wchnschr., 1906, XLIII, 642.

Two more frogs are now selected of about the same weight, and the dose to be injected is determined by the effect of the primary dose. If the heart, in the first frog, stopped in less than ten minutes, a relatively smaller dose is used, while if it did not stop in twenty minutes a larger dose should be employed. A sufficient number of frogs should be used so that four are found in which the hearts have stopped between seven and twenty minutes. The toxic value (V) of the drug is then determined in relation to the size of the frog (P) divided by the dose of the drug given (*d*) multiplied by the time it takes for the heart to come to a systolic standstill (*t*): $(V) = \frac{P}{d \times t}$. The "Vs," having been determined for each trial, are averaged and the amount found is considered as representing the value of the preparation of digitalis which has been tested. Focke considers that a good specimen of leaves should give a standard value of 5. He originally accepted all readings between ten and twenty-five minutes, but the method was modified later to time limits of seven to twenty minutes, as he found the results were much more uniform. If the heart stops in a shorter time than seven minutes, the differences in the values found are greater, or if over twenty minutes, he found the readings were much more variable, and the "Vs" obtained were very small. For this reason he dislikes Ziegenbein's and Frankel's methods, the former taking the least amount which will stop the heart which will usually occur anywhere between thirty-five and one hundred minutes (Ziegenbein says within two hours), while the latter takes as his end reaction the stoppage in from fifty to seventy minutes. Focke, therefore, prefers his method because it is more convenient, taking less time and because it is more exact, showing according to him only 8 per cent of error.

After Focke developed this method he considered a number of factors which influence the results of standardization and the variability of digitalis preparations. In order to determine the effect of season upon the frog's susceptibility, he examined leaves which had been very carefully dried and which would retain the same value at the various times of the year. He found that in the spring they gave an average value of 2.66; from the end of June to October, 4.36; and in October, 5. At this time he considered these variations due to differences in the nutrition of the frogs, but later researches[a] seemed to indicate that the temperature factor might also play some part in changing the V obtained.

Thus frogs operated on at a temperature of 17.5° to 18.5° in December showed a V of 5.5. At the same temperature the same drug had a V of only 4.7 the last of February, but at 19° to 21° gave nearly the original value. In June, to obtain this value, it was necessary to increase the temperature to from 22° to 23°, at 18.5°, the original December temperature, the value being only 3.8. At the same temperatures there seemed to be no difference in the reaction of frogs which had been captured recently and those kept in captivity for several months.

Physiological standardization may be carried out according to Focke, therefore, at any time during the year, but it is first necessary to standardize the animal against digitalis leaves of known value by regulating the temperature and then by cooling or heating the operating room to carry out the assay at the temperature found to show the standard value as arbitrarily adopted.

It has been recognized that wild plants are more toxic than cultivated, and in confirmation of this view Focke found the former to give a V of from 5 to 6, while the latter gave as Vs 2.6 and 3. The yearly variation in plants from the same district he found to be quite marked, the weakest giving a value of 4.3, while the strongest had a V of 8.5. It has been universally recognized also that the second-year plants just before flowering are more active than the first year. In agreement with this view Focke found that the second-year leaves collected in June have a toxic value 15 to 20 per cent higher than first-year leaves collected from plants grown in the same locality. Plants flowering in July seed in August, and at this time the leaves show the effect by a lessened toxicity, the relation being reversed, as at this time the first-year leaves are stronger. The effect of daylight was found to be very slight, even if the leaves were exposed to its influence for a year. As to the effect of the aging of the leaves he found that if they were collected and dried in the air in the ordinary way and preserved in nonair tight-flasks they will lose a large part of their activity.

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[a] Focke, Arch. d. Pharm., 1907, CCXLV, 646.

The greatest factor in this deterioration Focke considers to be the moisture content, and the changes which the leaves undergo during the first few months after their collection are largely due to this, as in the presence of moisture certain ferments present in the leaves are able to destroy some of the active constituents. To avoid this, he heats the leaves rapidly and strongly at a temperature not over 100° C., and when they are dried so as to contain only about 1½ per cent moisture they are preserved in lots of 50 grams in dark, air-tight jars. He found that if dried and preserved in this manner they lost only 5 per cent in V in a year. The last 1 per cent of moisture left in the leaves seemed to be bound in some manner, so that it was not active.[a] To show the importance of the moisture content factor, Focke believed that only about one-fifth of the differences in strength were due to all the other factors.

To avoid the variation due to the different localities, the leaves from all the different sources should be powdered after drying and mixed, so as to give a uniform product.

Siebert[b] reports the efforts of himself and Ziegenbein to determine a method by which digitalis and strophanthus preparations could be standardized biologically. The method adopted by them was the one developed by Arthur and Hans Meyer, which has been described earlier in the discussion of Ziegenbein's work.

In fifty experiments with digitalis leaves the most active preparation showed a toxicity of 0.03 gram for 100 grams frog weight, while the weakest required 0.075 gram to produce death. They, therefore, adopted as a standard strength for digitalis leaves 0.04 gram for 100 grams frogs.

The variation in strophanthus preparations was as follows: Weakest 0.06, strongest 0.008 c.c. for 100 grams frogs. The standard they adopted was 0.02 c.c.

An important chapter on the subject relating to the comparative study of the chemical and biological methods of assay of digitalis was contributed by Barger and Shaw[a] in 1904. Their investigations were carried out upon tinctures of digitalis prepared according to the British Pharmacopœia and obtained from various commercial sources. For the estimation of the digitoxin content they used Keller's method with slight modifications. For the biological experiments male frogs (Rana temporaria), weighing between 20 and 35 grams, were used almost exclusively, but comparative experiments were limited to those of nearly the same weight. The experiments were carried out in the early summer.

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[a] In this connection it is interesting to note that in 1867 Tourdes (Gaz. med. de Strasb., 1867, XXVII, 191) pointed out that the reason the digitalis preparations obtained in Strassburg were superior to those used elsewhere was because the leaves of the second year's growth were employed after having been selected carefully and dried first in the shade, and then in an oven at a temperature not over 40° C. The leaves were then preserved in tin or glass vessels away from the light and moisture.

[b] Siebert, Berl. klin. Wchnschr., 1903, XL, 813.

The tinctures were modified by evaporating a known amount over a hot-water bath and suspending the residue in hot water. The digitoxin employed in some of these experiments was dissolved in alcohol and later diluted with water so as to give it an alcoholic strength of 10 per cent, the digitoxin being injected in suspension. In all cases the drugs were injected into the dorsal lymph sac, the volume for decisive experiments being 1 c.c. The animals after injection were kept in a moist atmosphere and the time noted when the heart stopped, which always occurred within three hours, if at all.

After an estimation by chemical means of the digitoxin content in nine tinctures, they estimated the toxic value of the same tincture by the biological method described. The results obtained by the latter method show less variation between the tinctures than is usually found, as the strongest was only one and one-half times as toxic as the weakest.

They show that, on account of the slight toxic strength of digitalin and digitalein, a supposition exists that the toxicity of a tincture runs parallel to the digitoxin content (by Keller's method), but this is not correct, as they found that the ratio of toxicity of tinctures to the toxicity of the digitoxin content in them was as 1 to 3.5, 5, 3.7, 5.5, 6.9, 4.1, 4.1, 5, and 4.2.

The writers then estimated physiologically the toxicity of the water soluble active constituents, and this, added to the toxicity of the digitoxin, was compared to the total toxicity of the tincture. The results show that about 50 per cent of the tincture toxicity was still unaccounted for even when both water-soluble and insoluble constituents were considered.

In order to find the cause of this discrepancy they prepared a tincture from chaff and hay and added to this 0.04 per cent crystallized digitoxin. By biological methods 0.0375 per cent was demonstrated, while by Keller's method only 0.01 per cent was found. The cause of the difficulty was shown by further experiments to depend upon the fact that much of the digitoxin adheres to the resin which separates out when the alcohol is evaporated off from the tincture, this being lost in the chemical method of estimation, but appearing in the physiological.

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[a] Barger and Shaw, Pharm. J. & Tr., London, 1904, XIX, 249.

According to their results it appeared that 1 gram of the tincture would kill a 59-gram frog, but the water soluble active constituents would only kill a 15-gram frog. The 44 grams difference in frog weight therefore ought to be killed by the insoluble portion or digitoxin. The toxic value for the digitoxin for only a 17-gram frog had been demonstrated by the Keller method, however, leaving 27 grams of the toxicity of the digitoxin of the original tincture still unaccounted for and which probably remains in the resinous portion when Keller's method of determination is used. In other words, only about one-third to one-half of the digitoxin present is isolated by this method, and the authors conclude, therefore, that the only reliable method for the assay of digitalis tinctures is the physiological one.

Freund[a] studied the action of abyssinin and compared it with drugs of the digitalis groups, notably, digitalin, digitalein, digalen, the dialysate of digitalis, and strophanthin.

For the purposes of this investigation he used frogs and rabbits. With the former the heart was laid bare and attached to a suspension writing lever, thus obtaining tracings showing the progressive action of the drug. In some animals a solution of the drug was dropped directly on the heart, while in others it was injected into a lymph sac. In the case of rabbits Freund used the blood pressure changes as a method for comparing the relative action of the drugs.

In his study of the different heart drugs, Kakowski[b] examined a number of digitalis preparations, among them being the infusion and the tincture. He employed the isolated hearts of frogs (R. temporaria), rabbits, cats, and dogs and showed that the infusion acted more strongly upon some of the animals than the tincture.

Still another modification of the use of a frog's heart in estimating the activity of members of the digitalis group was adopted by Santesson[c] in his examination of the strength of different varieties of strophanthus seeds. In this method the drug was injected into a lymph sac of Rana temporaria. The animal was not tied down, but was held on its back in such a way that the contractions of the heart could be observed through the skin. In case it was necessary, a glass rod was passed into the esophagus so as to press the heart forward to facilitate the determination of its rate. Finally, to allow of exact timing of the appearance of the systolic standstill, the chest was opened and the organ observed directly. The standard dose employed by Santesson was that amount of the drug which would produce systolic standstill of the heart in thirty minutes, the dose being reckoned upon the basis of a 50-gram frog. Santesson's custom was to give a large dose at first, one that would be sure to kill, and on subsequent injections to diminish the amount until that dose was reached which would stop the heart in the required time. He points out that the biological standardization is not easy on account of the irregularity of the reaction of frogs. To avoid this as much as possible there should be taken into consideration the effect of season, species, body weight, temperature, condition of health, and, finally, a factor which can not be reckoned upon, viz, individual idiosyncrasy. The frogs employed by Santesson were captured in the autumn and kept in a cool place until they were used, in November and December. Males of about 50 grams weight were selected and kept in the examining room at least one hour before they were used. With these precautions Santesson showed that tinctures prepared from different varieties of strophanthus seeds varied in strength in the ratio of 1 to 4.

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[a] Freund, Zeitschr. Exp. Path. u. Therap., 1905, I, 557.

[b] Kakowski, Arch. Internat. de Pharmacod. Gand et Par., 1905, XV, 21.

[c] Santesson, Skandi. Arch. f. Phys., 1905, XVII, 389.

A second method of comparison was to perfuse the frog's heart, using William's apparatus, comparing the effect of the different drugs upon the pulse frequency, circulation rapidity (number of drops perfused per minute), and the pulse volume.

The results obtained by the two methods furnish a very interesting comparison.

Preparation.	Lethal dose for intact frog.	Lethal dose, isolated heart.
1	0.063	0.047
2	.10	.123
3	.22	.25
4	.10	.046
5	.064	.027
6	.051	.0925

Santesson calls attention to the fact that a certain parallelism exists in the results obtained by the two methods, but it is certainly very slight. He explains the lack of closer agreement as being due to the fact that the poison would not be so uniformly distributed in the fluids of the intact animal as in a fluid used to perfuse an isolated heart.

Dixon,[a] in a paper on the biochemical standardization of drugs, considers the frog as the most suitable animal for digitalis standardization. In employing them the time of year should be taken into consideration, as they are most active and vigorous in the summer and least active in the spring, but their sensitiveness to digitalis will not vary over 50 per cent during the year. Those selected should be males of about 25 grams weight and free from abnormal conditions. The tincture, diluted with an equal amount of water, is injected into the dorsal lymph sac and 6 minims of this diluted tincture should produce systolic stoppage of the heart in one hour. Dixon proposes this as a standard "unit," and if a certain tincture does not correspond to this strength the pharmacist should make the necessary calculations, so as to give the patient a uniform product. The writer thinks the method proposed above may be accurately controlled by experiments upon rabbits.

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[a] Dixon, Pharm. J. and Tr., London, 1905, p. 155; Manual of Pharmacology, 1906, p. 34.

In his Manual of Pharmacology (1906, p. 34), after discussing the frog method, Dixon says: "These drugs can be standardized more accurately by perfusing the isolated rabbit's heart with Ringer's solution and subsequently adding the drug." However, Sowton's results, referred to later, hardly confirm this statement.

Haynes[a] sought to determine the relative activity upon the heart of the three important members of the digitalis group—digitalis, strophanthus, and squills—using tinctures which he prepared very carefully according to the British Pharmacopœia. He standardized these by determining the smallest dose of each which, when injected into the dorsal lymph sac of a frog, would cause systolic stoppage of the heart. He also carried out comparative tests upon rabbits with these preparations, injecting the drugs invariably into the stomach and watching for changes in the blood pressure as well as for the toxic effects upon the heart. In addition he studied their relative strength upon the excised hearts of rabbits, employing a modification of the Langendorff method and compared especially the effect upon the rate and the quality of the heart beat.

In respect to the suggestion that, on account of the variation in the strengths of galenical preparations of this series, the active principles should be substituted for them in general practice, Haynes says that in their isolation much of their potency is lost and that they require standardization even more than the galenicals.

The Pharmacopœia of Norway (1870) directed that official preparations of digitalis should be made from the plant growing wild in Norway. Wang[b] undertook to determine whether the plants growing in that country offered any advantage as regards strength over those growing elsewhere.

He used frogs (Rana temporaria) and adopted Focke's method of assay described earlier. His experiments were carried out in Strassburg in October, 1905. The frogs were kept in a cool cellar, and the evening before they were needed they were placed in a cool room, being brought to ordinary room temperature two or three hours before they were used. The animals were injected with the 10 per cent infusion through the lateral lymph sacs into the two thigh lymph sacs. In this manner any escape of the drug was avoided when the chest was open. The heart was exposed as soon as the animal was injected and the time noted when systolic standstill appeared. Wang, in common with some other workers, found it hard to decide at just what time this end reaction occurred, and he therefore used the stoppage of the circulation as an end reaction. His results, using Focke's formula to give the toxic worth of the leaves, are very variable. In some of the tables, especially Table I, in which, in sixteen estimations of one preparation, he obtained values between 3.6 and 8.8, with an average of 5.2. The other tables are not nearly so irregular in their results, since in these he calculated his dose according to the body weight of the animal employed. Table III, for instance, only shows slight variations from 4.7 to 5.7, with an average worth for the specimen of 5.3.

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[a] Haynes, Bio-Chem. Jour., 1906, I, 63.

[b] Wang, Festschr. f. Olof Hammarsten, 1906.

Löwy[a] carried out experiments with infusions of digitalis to determine the effect of hydrochloric acid and pepsin, in the per cents in which they occur in the stomach, upon their action. He used 20-gram frogs (Rena temporaria), exposing the heart and after injecting 1 c. c. of the solution to be tested, noted the time of the appearance of systolic standstill.

Kochman[b] showed that five infusions of digitalis made from leaves obtained from different sources possessed different actions upon the blood pressure of dogs, only two of them producing the true digitalis effect of increased blood pressure and lessened pulse rate.

In a research to determine the uniformity of the preparations of digitalis and strophanthus which were upon the market Edmunds[c] examined seventeen preparations of the tinctures of digitalis and six of strophanthus. Although many of these were said to be physiologically standardized they showed great variation, two preparations (standardized) from one manufacturer having toxicities in the ratio of 1 to 2. Four nonstandardized preparations from one manufacturer varied as 1 to 4.

The method employed to standardize the drugs was the same as has been used in the pharmacological laboratory of the University of Michigan for a number of years and is essentially the same as that employed by Famulener and Lyons,[d] the only difference being that as an end reaction complete systolic stoppage of the heart is used, not only of the ventricle, as in the Famulener and Lyons method, but also of the auricles.

In his comparison of various tinctures of strophanthus which were on the market Hatcher[e] adopted Frankel's method of examination; that is, the amount necessary to produce systolic standstill of the frog's heart in about one hour, comparing the doses when calculated on the basis of 100 grams frog's weight. He also compared their action upon cats and dogs, these reacting by vomiting. He suggested on account of the regularity of the appearance of this symptom, that it might be thought of as an index of the potency of the drug but because of the variability of the reaction of the vomiting center he "considered it better to determine the amount necessary to produce systolic standstill which is, of course, promptly followed by death."

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[a] Löwy, Wien klin. Wchnschr., 1906, XIX, 1157.

[b] Kochman, Bull. Soc. de Med. de Gand., 1906, LXXIII, 95.

[c] Edmunds, J. A. M. A., 1907, XLVIII, 1744.

[d] Famulener and Lyons, Proc. Am. Pharm. Ass., 1902, L, 415.

[e] Hatcher, J. A. M. A., 1907, XLVIII, 1177.

In his article "Ueber die physiologische Wertbestimmung von Arzneimitteln" Gottlieb[a], after discussing the work of Frankel, Zeigenbein, and Bührer upon physiological standardization, mentions the results of his investigation upon the uniformity of digitalis leaves used in the clinics around Heidelberg, showing that they varied as much as fourfold. He calls attention to the necessity of a government institution where such preparations can be examined, a fact to which attention has been called by several earlier writers.

In order to obtain a convenient method of designating the strength of preparations he suggests the adoption of a standard "unit," using the term in the same way as it is used in connection with sera work. For such a unit he has adopted the following: "The smallest amount of the solution which will call forth systolic standstill of the heart of a R. temporaria of 30 grams weight in thirty minutes exactly." In an infusion freshly prepared from 1 gram of good digitalis leaf powder there must be 30 to 40 units; the strongest leaves may contain 120 units.

He further points out that such a comparison on the frog's heart is only possible when made with similar active constituents, such as we have in the leaves, infusion, and tincture of digitalis, but could not hold good with dissimilar constituents as, for instance, a comparison of strophanthus with digitalis. A further fact which he calls attention to is that, in the methods usually employed in which the heart is to be brought to a systolic standstill in a certain short time, a strong solution of a substance, absorbed with difficulty, would appear weaker than a weak solution of easily absorbable substances.

Sowton[b] undertook to examine the activity of twenty-six specimens of the tincture of digitalis by means of perfusing the hearts of rabbits. He chose animals weighing 3 or 4 pounds each, and, using the coronary vessels, perfused the right ventricle with a solution of digitalis of a strength of 1 to 200 made up in Locke's or Ringer's solution. From his results he grouped the preparations into two classes, "strong" and "weak," but later found that such a classification was impossible, as five of his specimens upon which seventeen experiments had been carried out were placed some in one group and some in the other, and yet they were probably alike, having been made in exactly the same manner from the same lot of leaves. The results indicated the practical worthlessness of the method, as rabbits' hearts evidently exhibit marked variations.

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[a] Gottlieb, München. med. Wchnschr., 1908, LV, 1265.

[b] Sowton, Brit. M. J., London, 1908, No. 2458, 310.

Lutzkaja[a] published a critical study of the Focke method of standardization which he thinks is preferable to the one-hour method which, according to his views, is too inexact. He carried out the experiments according to Focke's directions with the exception that he used September and October frogs.

In his first experiments, with 0.5 milligram doses of digitoxin injected into frogs of different weights, he obtained toxic values showing as great variations as from 1.9 to 8.3 with an average of 4.5. For his second experiments he estimated the doses so as to stop the heart in about ten minutes, and by this means the results were much more uniform, differing only 80 per cent. In a third experiment his results varied still less, only 65 per cent. His conclusion is that, in spite of some irritating individual variation, if a sufficient number of experiments are carried out useful results may be obtained. He urges as an objection to the "short-time methods" that they might not give sufficient time for all the poison to be absorbed and by this means a very active preparation, absorbed with difficulty, would appear weaker than a weak preparation containing easily absorbable constituents.

His results show that digitoxin and digitalinum verum do not give the same ratio on frogs as their relative doses appear to indicate that they possess in man; in some preparations of digitalis he suggests there might be a great deal of digitoxin, but in others not as much, and while they might show the same effect on frogs they would not on man. He therefore thinks such a condition would render the standardization of the leaves on frogs of little value.

Practically every worker upon this subject has employed the frog in some way or other as a means of standardizing the preparations of the drugs under consideration and the results obtained upon this animal have been confirmed in some cases by control experiments carried out, usually upon the blood pressure, upon the higher animals. However, in 1908 Reed and Vanderkleed[b] introduced a method, using guinea pigs, as they object to the frog because the reaction of this animal is very irregular, being affected by the season of the year, the species, etc. They also point out that the action upon the heart is a toxic effect, a determination of the lethal dose, and therefore they substitute for the toxic dose in frogs the toxic dose in guinea pigs. To make the assay they prepare their tinctures by evaporating off the alcohol and diluting the residue with water. Progressively increasing doses are then injected subcutaneously into guinea pigs of 240 grams weight until the amount is found which will kill the animal in one and one-half to two hours. Their standard dose then, which they confirm by injecting like amounts into five or six animals, is that amount which would kill a guinea pig of 240 grams weight in one and one-half to two hours. They carried out a series of experiments to compare the digitoxin content of tinctures with the physiological activity. These results are tabulated on page 9.

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[a] Lutzkaja, Arch. internat. de Pharmacod, Gand et Par., 1908, XVIII, 77.

[b] Reed and Vanderkleed, Am. J. of Pharm., 1908, LXX, 110.

EXPERIMENTAL WORK.

As will be seen from a study of the literature that has been given there are essentially three methods used for the biological assay of the members of the digitalis series, the others being merely modifications departing more or less widely from what we may consider the primary groups. These may be classified as follows: A toxic method, in which frogs, guinea pigs, or some of the higher animals are used; second, a method using the frog's heart, which is perfused in some cases while in others it is simply exposed and systolic standstill is watched for (this in Focke's method is reached in from seven to twenty minutes, while in others a longer interval of one or two hours is allowed); the third class includes those methods which aim at a comparison by means of the relative effects upon the blood pressures of some of the higher animals.

One of the objects of the present study has been to examine into the results of these various methods to find if possible if one possessed a marked advantage over the others, and, most important of all, to see whether the different methods would give anything approaching uniform results in a comparative study of a number of digitalis preparations, some of which have been made according to the pharmacopœial requirements, while others had been manufactured according to some special method devised by individual pharmaceutical houses. A study of these preparations constitutes an important part of the work, but was not the primary object of the research.

With these objects in view we have examined nine preparations of digitalis, namely: Fluid extracts prepared according to the U. S. Pharmacopœia, VIII, by Messrs. Parke, Davis & Co., Detroit; Sharpe & Dohme, Baltimore; Nelson, Baker & Co., Detroit; Hance Brothers & White, Philadelphia. In addition to these we examined several proprietary preparations as follows: Three specimens of Digitalone, prepared by Parke, Davis & Co.; a fat-free tincture of digitalis (Digitol), prepared by H. K. Mulford & Co., Philadelphia; Digitalis, Specific Medicine, manufactured by Lloyd Brothers, Cincinnati; a Concentrated Tincture of Digitalis (1 to 4), prepared by Burroughs, Wellcome & Co., London, and finally a purified "Normal Tincture," prepared by William S. Merrell & Co., Cincinnati.

Digitalone is said to be a "nonalcoholic permanent solution of digitalis" corresponding in strength to the U. S. Pharmacopœia tincture and physiologically standardized. The manufacturers state that

Digitol is a preparation of tincture strength made from digitalis leaves from which the fixed and volatile oils have been removed. It is "assayed, tested physiologically," and is standardized to contain 0.025 gram digitoxin in 100 c. c. Digitalis (Specific Medicine) is made from fresh digitalis leaves, and is of such a strength that 480 grains of the drug are contained in each fluid ounce of "80 per cent absolute alcohol." Concentrated Tincture of Digitalis (1 to 4) is said to be physiologically standardized and is of such a strength that 1 c. c. added to 4 c. c. dilute alcohol makes a preparation corresponding in strength to the U. S. Pharmacopœia tincture. Purified Normal Tincture is said to be a standardized neutral tincture of prime digitalis freed from irritating fats and oils.

It was desired for the sake of comparison that all experiments with these various preparations be carried out upon the basis of the official tincture strength. No difficulty was experienced in doing this except with Merrell's Normal Tincture, the name of which might suggest that it was a 10 per cent preparation, but for reasons to be considered later, the dose prescribed, etc., it was considered to correspond to a fluid-extract strength. Accordingly a measured quantity of each preparation (excepting Digitol and Digitalone) was taken and after evaporating off the alcohol over a water bath it was diluted to ten times the original volume with physiological salt solution. Digitol was evaporated to free it from the contained alcohol and then diluted to the original volume only. Digitalone, being nonalcoholic, was not evaporated excepting in some experiments when it was concentrated in order to avoid such a large bulk of fluid as the doses given required. In all cases resinous bodies, precipitated by the evaporation of the alcohol and the addition of the salt solution, were injected in suspension to insure the introduction of any active bodies which might be carried down by their precipitation.

For the sake of uniformity the following abbreviations have been adopted to designate the above preparations in all of the tables which follow:

Digitol, bottle No. 1 = Mulford No. 1.
Digitol, bottle No. 2 = Mulford No. 2.
Normal Tincture Digitalis = Merrell.
Concentrated Tincture Digitalis = B., W. and Co.
Specific Medicines, Digitalis = Lloyd Bros.
Nelson Baker and Co., Fluid Extract Digitalis = N., B. and Co.
Sharpe and Dohme, Fluid Extract Digitalis = S. and D.
Hance Bros. and White, Fluid Extract Digitalis = H. B. and W.
Parke, Davis and Co., Fluid Extract Digitalis = P., D. and Co.
Parke, Davis and Co., Digitalone bottle No. 1 = Digitalone No. 1.
Parke, Davis and Co., Digitalone bottle No. 2 = Digitalone No. 2.
Parke, Davis and Co., Digitalone bottle No. 3 = Digitalone No. 3.

MINIMUM LETHAL DOSE METHOD OF ASSAY.

The method adopted for examination and the animals employed by us as illustrating the toxic methods of assay were as follows:

Mice.—White mice, kept under the same conditions and varying in weight from 15 to 25 grams, were used. The drug was injected hypodermically beneath the skin of the back, the dose being calculated at so many milligrams per gram of body weight and so diluted that a dose of 1 c. c. was not exceeded in any case. The mice were then watched and the death or survival of the animal noted. The data thus obtained (as were the results in all subsequent series of experiments) were confirmed by injecting a second series of mice several days later. The results of these tests are given in Table I.

TABLE I.—*Determination of the minimum lethal dose per gram body weight for white mice; subcutaneous injection.*

[Survived = −; dead = +.]

MULFORD NO. 1.

Number of animals used.[a]	Dose.	Results.
	mgm.	
1	2	−
4	3	−
5	4	+
3	5	+
3	6	+

MERRELL.[b]

1	3	−
1	4	−
2	5	−
1	5	+
3	6	+
2	7	+

B. W. AND CO.

3	3	−
2	4	+
1	5	+
1	6	+
2	7	+

H. B. AND W.

3	6	−
1	7	−
3	7	+
2	8	+
1	9	+

DIGITALONE NO. 1.

1	10	−
1	19	−
1	20	−
1	22	+
1	25	+
1	28	+
1	30	+

LLOYD BROS.

Number of animals used.[a]	Dose.	Results.
	mgm.	
2	18	−
2	19	−
2	20	−
1	20	+
2	21	−
1	21	+
1	22	−
2	22	+
3	23	−
2	23	+
2	24	−
4	24	+

N. B. AND CO.

3	7	−
2	8	−
2	8	+
3	9	+
1	11	+

S. AND D.

2	6	−
2	7	−
4	8	−
1	8	+
2	9	−
3	9	+
1	10	−
3	10	+

P., D. AND CO.

2	3	−
3	4	+
3	4	−
2	5	+
3	5	−
2	6	+
3	6	−
2	7	+
3	7	−
4	8	+

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[a] The heading "Number of animals used" refers to the number of animals which received the dose indicated in the second column.

[b] Dose calculated on the basis that preparation was of fluid extract strength.

TABLE I.—*Determination of the minimum lethal dose per gram body weight for white mice; subcutaneous injection*—Continued.

SUMMARY.

Minimum lethal dose for mice per gram body weight.

Preparation.	Milligrams.
Mulford (digitol)	4
B. W. and Co. (concentrated tincture)	4
Merrell (normal tincture)	6
H. B. and W. (fluid extract)	7
P., D. and Co. (fluid extract)	8
N., B. and Co. (fluid extract)	9
S. and D. (fluid extract)	9
Digitalone No. 1 (P., D. and Co.)	22
Lloyd Bros. (specific medicine)	24

Guinea pigs.—Guinea pigs of about the same weight were injected beneath the skin of the abdomen with the drugs to be tested. The dose was reckoned according to the weight of the animal and the preparations were so diluted as to make the bulk of the fluid injected between 1 and 2 c. c. The results of these experiments are recorded in Table II.

TABLE II.—*Determination of the minimum lethal dose for guinea pigs—subcutaneous injection.*

[Survived = −; dead = +.]

MULFORD NO. 1.

Number of animals used.[a]	Dose.	Result.
	mgm.	
1	0.56	−
1	.625	−
2	.65	−
1	.70	−
1	.70	+
1	.75	+
1	.80	+

MERRELL.

Number of animals used.[a]	Dose.	Result.
1	0.40	−
2	.50	−
4	.50	+
3	.60	−
5	.60	+
1	.65	+
2	.70	+

B. W. AND CO.

Number of animals used.[a]	Dose.	Result.
1	0.25	−
2	.30	−
1	.35	−
2	.35	+
1	.40	+
1	.55	+
1	.68	+

LLOYD BROS.

Number of animals used.[a]	Dose.	Result.
	mgm.	
2	0.70	−
2	.80	−
2	.80	+
2	.90	−
3	.90	+
1	1.00	−
2	1.00	+
1	1.50	+
1	2.00	+

DIGITALONE NO. 1.

Number of animals used.[a]	Dose.	Result.
1	2.00	−
1	4.00	−
1 [b]	5.00	+

N., B. AND CO.

Number of animals used.[a]	Dose.	Result.
1	0.50	−
1	.70	−
2	.80	−
2	.80	+
3	.90	+
2	1.00	+
1	1.20	+

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[a] This heading refers to the number of animals which received the dose indicated in the second column.

[b] Guinea pig, weight 250 grams, injected with 12.5 c. c., digitalone No. 1. No digitalis symptoms whatever appeared, the animal passing at once into a comatose condition. The respiration became weaker and finally ceased. Cause of death (?).

TABLE II.—*Determination of the minimum lethal dose for guinea pigs—subcutaneous injection*—Continued.

DIGITALONE NO. 3.

Number of animals used.	Dose.	Result.
	mgm.	
1	0.90	–
1	1.50	[a]–

H. B. AND W.

Number of animals used.	Dose.	Result.
1	0.30	–
2	.40	–
2	.50	+
1	.70	+
1	1.00	+

P., D. AND CO.

Number of animals used.	Dose.	Result.
	mgm.	
1	0.50	–
1	.60	–
1	.70	–
2	.70	+
2	.80	+

S. AND D.

Number of animals used.	Dose.	Result.
1	0.70	–
3	.80	–
1	.80	+
1	.90	–
4	.90	+

[a] No symptoms.

Digitalone No. 3 was used in injecting two guinea pigs, one receiving 0.9 milligram and the other 1.5 milligrams per gram weight. Neither of these animals showed any symptoms whatever excepting that they were somewhat less active than normal.

SUMMARY.

Preparation.	M. L. D.[a]
	mgm.
B. W. and Co	0.35
H. B. and W	.50
Merrell	.60
Mulford No. 1	.70
P., D. and Co	.70
N., B. and Co	.90
S. and D	.90
Lloyd Bros	.90
Digitalone No. 1	5.00(?)
Digitalone No. 3	[b] 1.50

[a] M. L. D.=minimum lethal dose.

[b] Lived.

Cats.—The toxic dose was determined for these animals in the course of the blood-pressure experiments. After the initial injection of 1 c. c. of the 10 per cent solution, further injections of 0.5 c. c. each were administered at intervals of not less than 5 minutes, artificial respiration being maintained so that the toxic action was due to an effect upon the heart rather than upon the medulla. This was thought desirable because in some of the earlier experiments the respiration was observed to have failed, although a good circulation was being maintained, while in others the animal would make apparently normal respiratory efforts after the heart had stopped and the blood pressure had fallen to zero. The toxic doses were compared on the basis of the number of milligrams of the drug used per kilogram of body weight of the animal. The results of this series of experiments and the average for each drug used are given in the last two columns of Table III.

Table III.—*Determination of blood pressure and minimum lethal dose for cats, intravenous injection. Fluid extracts diluted to 10 per cent strength.*

	Heart rate.	Blood pressure.	Percentage increase in blood pressure.	Lethal dose.	Lethal dose per kilogram, body weight.
N., B. AND CO.					
August 4, 1908, cat, 3,800 grams:				*c. c.*	*mgm.*
Normal	126	55			
After 1 c. c.	150	122	122	7	184
September 9, 1908, cat, 3,780 grams:					
Normal	90	38			
After 1 c. c.	90	60	58	6	158
September 9, 1908, cat, 2,930 grams:					
Normal	120	36			
After 1 c. c.	120	78	116	7	235
Average			99		192
MULFORD NO. 2.					
August 6, 1908, cat, 1,380 grams:					
Normal	162	60			
After 1 c. c.	186	140	133	2	145
August 6, 1908, cat, 1,870 grams:					
Normal	192	68			
After 1 c. c.	186	86	27	4	214
September 4, 1908, cat, 3,250 grams:					
Normal	198	66			
After 1 c. c.	174	84	27	7	215
September 11, 1908, cat, 2,870 grams:					
Normal	180	94			
After 1 c. c.	180	158	68	6	208
Average			64		195
DIGITALONE NO. 2.					
August 5, 1908, cat, 1,800 grams:					
Normal	174	66			
After 1 c. c.	168	100	52	9	500
September 9, 1908, cat, 2,500 grams:					
Normal	150	38			
After 1 c. c.	160	60	58	15	600
Average			55		550
B. W. AND CO.					
August 5, 1908, cat, ——:					
Normal	198	80			
After 1 c. c.	210	104	30		
August 7, 1908, cat, 1,150 grams:					
Normal	180	39			
After 1 c. c.	186	74	90	2	174
September 4, 1908, cat, 3,500 grams:					
Normal	138	50			
After 1 c. c.	138	66	32	5	143
Average			51		158
P., D. AND CO.					
August 5, 1908, cat, 1,600 grams:					
Normal	114	40			
After 1 c. c.	126	55	38	3	187
September 5, 1908, cat, 3,220 grams:					
Normal	174	56			
After 1 c. c.	162	76	36	5	155
Average			37		171
S. AND D.					
August 7, 1908, cat, 1,150 grams:					
Normal	216	94			
After 1 c. c.	210	112	19	2	174
September 5, 1908, cat, 1,690 grams:					
Normal	144	40			
After 1 c. c.	114	64	60	5	296

Table III.—*Determination of blood pressure and minimum lethal dose for cats, intravenous injection. Fluid extracts diluted to 10 per cent strength*—Continued.

	Heart rate.	Blood pressure.	Percentage increase in blood pressure.	Lethal dose.	Lethal dose per kilogram, body weight.
S. AND D.—continued.					
September 8, 1908, cat, 2,880 grams:				*c. c.*	*mgm.*
Normal	158	56			
After 1 c. c	162	74	32	6	208
September 8, 1908, cat, 3,800 grams:					
Normal	162	64			
After 1 c. c	156	84	31	11	289
Average			35		242
H. B. AND W.					
August 4, 1908, cat, 2,400 grams:					
Normal	162	58			
After 1 c. c	204	100	72	4	166
September 10, 1908, cat, 3,050 grams:					
Normal	220	68			
After 1 c. c	190	78	15	7	229
September 10, 1908, cat, 3,900 grams:					
Normal	120	50			
After 1 c. c	120	62	24	9	231
September 11, 1908, cat, 3,620 grams:					
Normal	130	70			
After 1 c. c	140	85	21	9	249
Average			33		219
MERRELL.					
August 4, 1908, cat, 3,600 grams:					
Normal	138	54			
After 1 c. c	132	70	30	7	194
September 12, 1908, cat, 2,540 grams:					
Normal	200	90			
After 1 c. c	210	104	16	7	275
September 16, 1908, cat, 2,800 grams:					
Normal	140	54			
After 1 c. c	140	72	33	8	286
Average			26		252
DIGITALONE NO. 3.					
August 6, 1908, cat, 1,600 grams:					
Normal	138	70			
After 1 c. c	120	84	20	4	250
August 6, 1908, cat, 1,930 grams:					
Normal	120	46			
After 1 c. c	108	60	31	6	310
Average			26		280
LLOYD BROS.[a]					
August 5, 1908, cat, 2,000 grams:					
Normal	168	66			
After 1 c. c	168	84	27	8	400
September 11, 1908, cat, 2,730 grams:					
Normal	130	52			
After 1 c. c	130	63	21	13	476
Average			24		438
DIGITALONE NO. 1.					
August 5, 1908, cat, 1,800 grams:					
Normal	180	68			
After 1 c. c	210	48	[b] 29		
September 5, 1908, cat, 3,220 grams:					
Normal	160	60			
After 1 c. c	190	50	[b] 17		
Average			[b] 23		

[a] Specific medicine. Evaporated at room temperature and diluted to 10 per cent strength with salt solution.

[b] Per cent fall.

TABLE III.—*Determination of blood pressure and minimum lethal dose for cats, intravenous injection. Fluid extracts diluted to 10 per cent strength*—Continued.

SUMMARY OF TOXIC DOSES FOR CATS (AVERAGES).

Preparation.	Milligrams.
B. W. and Co	158
P., D. and Co	171
N., B. and Co	192
Mulford No. 2	195
H. B. and W	219
S. and D	242
Merrell	252
Digitalone No. 3	280
Lloyd Bros	438
Digitalone No. 2	550

Frogs.—Finally the toxic dose on frogs was found according to Houghton's[a] method (see p. 16). The frogs (R. pipiens, Schreber, Syn. R. virescens and halecina) were unpacked as soon as they arrived and placed in a large cage through the end of which flowed fresh water. They were only brought to the experimental room when needed and injected late in the afternoon. They were then placed in separate jars and early the following morning the death or survival of each animal was noted. The results of experiments in which the lethal doses of four of the digitalis preparations were determined are given in Table IV.

TABLE IV.—*Determination of the minimum lethal dose for Rana pipiens, twelve-hour method.*

[Survived = –, dead = +. Dose per gram body weight.]

MULFORD NO. 2.

Series.	Dose.	Result.
	c. c.	
1	0.016	–
	.018	–
	.020	+
2	.019	+
	.020	+
	.020	–
3	.020	+
	.020	+
	.020	–

P., D. AND CO.

Series.	Dose.	Result.
	c. c.	
1	0.024	–
	.026	–
	.028	–
2	.030	+
	.032	–
	.034	+
3	.029	+
	.030	+
	.032	+
4	.029	+
	.029	+
	.029	+

B. W. AND CO.

Series.	Dose.	Result.
1	0.010	–
	.012	–
	.014	–
2	.014	+
	.016	+
	.018	+
3	.015	+
	.015	+
	.015	+

S. AND D.

Series.	Dose.	Result.
1	0.025	–
	.026	+
	.027	–
2	.026	–
	.027	+
	.028	+
3	.027	+
	.027	+
	.027	–

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[a] Houghton, J. Am. Med. Ass., Chicago, 1898, XXXI, 959.

TABLE IV.—*Determination of the minimum lethal dose for Rana pipiens, twelve-hour method*—Continued.

SUMMARY.

Preparation.	M. L. D.[a]
	c. c. mgm.
B. W. and Co	0.015=1.5
Mulford No. 2	.020=2.0
S. and D	.027=2.7
P., D. and Co	.029=2.9

[a] M. L. D.=minimal lethal dose.

Factors modifying effect.—Our experiences with the various factors which are supposed to influence the reaction of the frogs may be briefly summarized here.

Weight.—In regard to the weight of the animal many workers have apparently very largely ignored this factor, while others have made only an approximate allowance for it. For instance, Focke says to use frogs weighing from 25 to 30 grams, while others merely say "large" frog or "medium" or "small," as the case may be. In our experience, which covers some years, we have always weighed the animals fairly accurately, within the limits of 1 gram, and then calculated the dose per gram body weight.[a] This seems not to be absolutely necessary, judging from the reports of several writers quoted, but we think that it can not help but insure greater uniformity in the results, especially when there are certain factors such as idiosyncrasy which can not possibly be allowed for.

Sex.—Our experiences agree with those of Focke that unless it is in the springtime there is no particular difference in the reaction obtained from male and female frogs.

Season.—The effect of the season of the year upon the conditions of the frogs seems to be pretty generally recognized. Both Moschkowitsch and Dixon point out that the frogs are most active in the spring. The question has been most fully considered by Focke, who says that on account of the seasonal variations only summer frogs should be employed, as they not only react differently at this time but there is very much less variation in their reaction. According to a later research,[b] if they are to be used at any other season, they should themselves be standardized by testing them against a digitalis preparation of known value and using the temperature factor which will give the preparation its standard "V" as previously determined. The introduction of this factor, however, we believe to be entirely unnecessary excepting that comparative experiments should be carried out at the same temperature. That a given preparation should show a worth of 5 at one time and only 3 at another seems of very little importance if its keeping qualities are known to be good. All that is necessary is to keep such a preparation in stock and, at any time when an assay is to be made, to redetermine its activity and then make the unknown conform to the value found. The only essential is that the known and the unknown digitalis preparation be tested at the same temperature.

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[a] In our former work we used as a standard for comparison doses calculated on the basis of a 20-gram frog, but in this work we have compared the preparations upon the basis of 1-gram body weight as being simpler.

[b] Focke, Arch. d. Pharm., 1907, CCXLV, 646.

Focke[a] carried out a series of experiments especially designed to show the effect of season. (The temperature factor probably played some part in the results.) He employed a digitalis powder which had been very thoroughly dried and carefully preserved. During April, May, and the early part of June the average of all experiments gave the powder a worth of 2.66. From the last of June to the end of September a V of 4.36; during October, a V of 5, and on November 20 a V of 3.3. These variations he ascribes to the nutrition of the frogs which is lowest in the spring and gradually improves during the summer, reaching its highest point just before the winter sets in, when it falls off sharply until it reaches the low point in the spring. He also says in a later paper that in the spring the frogs react weakly, and this is supported by the results of experiments in which he shows that the temperature must be increased in order to obtain the same values for the same preparation as at other seasons. His tables show that reacting weakly the frogs require a larger dose of the drug or an increase in the temperature of the operating room to produce the same effect and therefore give a lower V to the specimen of leaves examined.

The effect of a lowered nutrition in increasing the resistance to the drug is rather surprising, especially as our experiments, while not as complete as Focke's, show exactly the opposite effect, viz, that in the summer when the animals are most vigorous they require a larger dose to produce systolic standstill of the heart. We worked with a different variety of frogs from those Focke employed, but it would hardly be expected that this factor should make such a fundamental difference. Our experience may be summarized as follows:

The U. S. Pharmacopœia tincture examined by Edmunds[b] in 1907 required a dose of from 0.16 to 0.20 c. c. to produce systolic standstill in one hour. In experiments carried out in August, 1908, frogs belonging to the same species (R. pipiens) and of the same size required 0.40 to 0.50 c. c. to produce the same effect.

To be still more exact, in April, 1907, a Parke, Davis and Co. tincture required 0.15 c. c. to produce the end reaction described above; in 1908 one of their fluid extracts made to correspond to tincture strength required 0.42 c. c. As these preparations are both physiologically assayed to a definite strength, the difference must be ascribed to the variability of the reaction of the frogs. As a means of demonstrating the uniformity of these two preparations mentioned, we might say that the 1907 tincture corresponded in strength to a tincture made in the laboratory at Ann Arbor from leaves obtained from Parke, Davis and Co., while their 1908 preparation is practically the same strength as U. S. Pharmacopœia fluid extracts made by other firms and which were examined at the same time, the results being given in the table on page 44.

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[a] Focke, Arch. d. Pharm., 1903, CCXLI, 678. Ther. d. Gegenw., 1904, XLV, 251.

[b] Edmunds, J. Am. Med. Ass., Chicago, 1907, XLVIII, 1744.

In striking contrast to these views are the results of some of Houghton's[a] assays, which were carried out to ascertain if a standard tincture would deteriorate in a year if kept under proper conditions. His two tables, which we give below, show not only that the standard preparations do not deteriorate, but also, what is equally important and interesting, that frogs react exactly the same at all times of the year.

TABLE III A.—*Standard strophanthus.*

Date.	Toxic dose.
July 6, 1897	0.00015
October 19, 1897	.00015+
December 20, 1897	.00015
February 23, 1898	.00015−
April 25, 1898	.00015

TABLE III B.—*Concentrated strophanthus.*

Date.	Toxic dose.
July 28, 1897	0.0000037
November 10, 1897	.0000037

Ziegenbein also did not think that season made much difference in the susceptibility of frogs. However, it may be said that any errors due to differences in the reaction of the frogs dependable upon season can be avoided, in the first place, by standardizing the frogs with reference to the temperature of the operating room after the manner of Focke, and, second and more simply, these differences may be avoided by always assaying a standard solution of digitalis, which is kept under proper conditions at the same time that the unknown solution is examined.

FROG-HEART METHODS OF ASSAY.

According to our classification, the second group is concerned with an action upon the frog's heart as a means of measuring the comparative strengths of the different preparations. In these methods the end reaction is the stoppage of the frog's heart in systole, the chief differences between the various methods being dependant upon variations in the time factor.

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[a] Houghton, J. Am. Med. Ass., 1898, XXXI, 959.

The one-hour method.—In this method the frogs are secured and kept in the manner already described, weighed, and such a dose is injected that the heart will be found in complete systolic contraction at the end of exactly sixty minutes. The drug, properly diluted so as to make a volume of 0.5 to 1 c. c., is injected into the anterior lymph sac by means of a glass pipette. Shortly before the hour is up the frog is pithed, tied to a frog board, and the heart is exposed in the usual manner. If the heart is still beating, the dose has been too small and must be increased in subsequent trials. In the first series of frogs doses are chosen with wide limits, which in a second and third series of animals are narrowed down until the smallest amount of the drug which will produce systolic standstill in one hour is found. Usually three series of frogs are sufficient to assay one preparation, but in case of any irregularity in the reaction of any of the frogs a fourth or even a fifth series may be necessary. The results of our assay of the different preparations are given in Table V.

TABLE V.—*Determination of the systolic stoppage of the heart of Rana pipiens in one hour—Drug injected into abdominal lymph sac. The doses given are in cubic centimeters per gram body weight.*

MULFORD NO. 1.

Number of animals used.	Dose.	Result.
	c. c.	
1	0.009	—
1	.01	—
3	.011	—
1	.0115	—
3	.012	+
1	.014	+
1	.016	+

DIGITALONE NO. 3.

2	0.035	—
1	.037	—
1	.038	—
1	.039	+
1	.040	—
2	.040	+
1	.041	—
1	.042	+

S. AND D.

1	0.025	—
1	.026	—
1	.026	+
1	.027	—
3	.027	+
1	.028	—
3	.028	+
1	.029	+

B. W. AND CO.

Number of animals used.	Dose.	Result.
	c. c.	
1	0.018	—
1	.019	—
1	.020	[a] +
1	.020	+
1	.021	+
1	.022	+

LLOYD BROS.

1	0.027	—
3	.028	—
3	.029	—
4	.030	+

DIGITALONE NO. 2.

1	0.025	—
1	.032	—
1	.040	—
1	.090	—
1	.105	—
1	.120	([b])

MULFORD NO. 2.

1	0.014	—
2	.015	—
1	.016	—
4	.016	+
1	.017	+

[a] Diastole.

[b] Diastole, later beating.

TABLE V.—*Determination of the systolic stoppage of the heart of Rana pipiens in one hour—Drug injected into abdominal lymph sac. The doses given are in cubic centimeters per gram body weight*—Continued.

MERRELL.

Number of animals used.	Dose.	Result.
	c. c.	
2	0.022	−
2	.023	−
1	.023	+
2	.024	−
3	.024	+
5	.025	+
1	.026	+

H. B. AND W.

Number of animals used.	Dose.	Result.
	c. c.	
2	0.022	−
1	.023	−
2	.024	−
2	.025	+
1	.026	−
2	.026	+
1	.027	+

N., B. AND CO.

1	0.020	−
2	.021	−
1	.021	+
1	.022	+
1	.023	+
1	.025	+

P., D. AND CO.

1	0.018	−
1	.019	−
1	.020	−
2	.020	+
2	.021	−
3	.021	+
3	.022	+

SUMMARY.

Preparation.	Dose.
	c. c. mgm.
Mulford No. 1	0.012=1.2
Mulford No. 2	.016=1.6
B. W. and Co	.020=2.0
P., D. and Co	.021=2.1
N., B. and Co	.022=2.2
Merrell	.024=2.4
H. B. and W	.025=2.5
S. and D	.027=2.7
Lloyd Bros	.030=3.0
Digitalone No. 3	.040=4.0
Digitalone No. 2	....

"Focke" method.—The second method upon the frog's heart which we employed was that of Focke, modified, however, in one or two important particulars. In the first place, we pithed all the frogs, while Focke does not do this until the experiment is over. It is absolutely essential that this should be done for purely humanitarian reasons, and if this operation interferes with the accuracy of his method then the method can not be used. As a matter of fact, this was done very carefully so as not to lose any blood, and the piece of sharpened wood was left in the brain cavity so as to prevent hemorrhage. The other modification was patterned after Wang, who found it was hard to determine when the ventricle may be said to have come to a permanent systolic standstill. We found exactly the same difficulty, and so adopted Wang's modification of watching for the stoppage of the circulation. Wang does not mention how he determines this, but the method we used was to watch with the microscope the stoppage of the circulation in the web of the foot. This itself is not always satisfactory, because in some animals, due to

some unknown condition, very little movement will be found in the blood in the web vessels. The most important factor in the condition of the peripheral circulation seemed to be whether any hemorrhage whatever had occurred during the operation. If even a drop of blood had been lost its loss appeared to be shown by the poor circulation in the web. An important precaution we adopted was, after selecting the field which showed the most favorable circulation, to note the direction of the flow in the main streams. If this was not done, after the heart stopped or had become very weak the blood would fre-frequently flow in the reverse direction and continue doing so for a long time. If it was not known that the blood was no longer going in the normal direction it would be supposed that the circulation had not ceased.

In most cases it was very hard to tell exactly when the circulation did actually stop, because when the heart became weak the blood would stop flowing for a short period, to be followed by a renewal of the flow just as soon as enough pressure was obtained in the supplying arteries to overcome peripheral resistance. Both end reactions, either stoppage of the heart or of the circulation, are very indefinite, in our opinion, but probably if a sufficient number of experiments are carried out a fairly definite idea of the relative strength of the preparations can be obtained.

It is very essential in this method if a comparison of different preparations is to be carried out not merely to choose any dose which will stop the heart at any time between seven and twenty minutes, because quite different values are obtained if a dose is given which will stop all the hearts in eight minutes or in say eighteen minutes. As we found, and as Focke shows in his tables, the shorter times give higher values, so that in comparing two preparations it would be necessary to have the hearts stop at some average time, ten or twelve minutes, or to so regulate the doses that two hearts will stop in about eight minutes, two in twelve or thirteen, and two in eighteen. We adopted this rule and in this way a fairly good average is attained. However, even with these precautions, we believe that this method allows of greater variations and inaccuracies than any other method we employed. The results of the determination of the toxic dose of the different digitalis preparations by this method are given in Table VI.

TABLE VI.—*Determination of toxic dose for Rana pipiens, Focke method—drug injected into leg lymph sacs.*

Time is noted when circulation in web of foot stops. Dose is calculated per gram body weight, animals weighing between 25 and 30 grams each

MULFORD NO. 1.

Dose.	Time.	Value (V).
c. c.	*Min.*	
0.020	18	2.8
.020	18	2.8
.020	14	3.6
.0225	9	4.9
.0225	12	3.7
.023	11	3.9
.025	9	4.4
.025	9	4.4
Average	....	V=3.8

MERRELL.[a]

Dose.	Time.	Value (V).
0.035	20	1.4
.035	15	2.0
.036	16	1.8
.0375	13	2.0
.0375	13	2.0
.038	12	2.2
.040	12	2.8
.040	10	2.5
Average	....	V=2.1

P., D. AND CO.

Dose.	Time.	Value (V).
0.0375	17	1.6
.0375	14	1.9
.040	13	1.9
.040	14	1.8
.040	14	1.8
.0425	11	2.1
.0425	10	2.4
.0425	11	2.1
.045	10	2.2
.045	11	2.0
.045	8	2.8
.045	9	2.5
Average	....	V=2.1

LLOYD BROS.

Dose.	Time.	Value (V).
0.035	15	1.9
.0375	13	2.1
.040	12	2.1
.040	10	2.5
.0425	12	1.9
.045	8	2.9
.045	9	2.4
Average	....	V=2.3

N., B. AND CO.

Dose.	Time.	Value (V).
c. c.	*Min.*	
0.015	27	....
.020	18	2.7
.020	17	2.9
.0225	10	4.4
.025	20	2.0
.026	12	3.2
.027	15	2.5
.030	13½	2.5
.035	10	2.9
Average	....	V=2.9

H. B. AND W.

Dose.	Time.	Value (V).
0.0325	14	2.2
.0325	15	2.0
.033	13	2.3
.035	12	2.4
.035	11	2.6
.0375	8	3.3
.0375	8	3.3
.038	7	3.7
.040	11	2.3
.040	7	3.6
Average	....	V=2.7

B. W. AND CO.

Dose.	Time.	Value (V).
0.03	13	2.6
.03	16	2.2
.03	12	2.7
.035	15?	1.9
.035	10	2.8
.035	9	3.2
.04	8	3.1
.04	7½	3.3
Average	....	V=2.7

S. AND D.

Dose.	Time.	Value (V).
0.036	16	1.8
.038	10	2.6
.038	12	2.2
.040	11	2.3
.045	10	2.2
.045	9	2.5
.045	8	2.8
.050	7	2.8
Average	....	V=2.4

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[a] Calculated as of fluid extract strength.

Digitalone No. 1. Frog, weight 41 grams, injected with 0.5 c. c. per gram body weight. Five hours later heart rate was 16, weak contraction but dilating well.

Digitalone No. 2. Frog, weight 40 grams, injected with 0.5 c. c. per gram body weight. Five hours later heart still beating, rate 17, contractions weak, but dilating fairly well.

Digitalone No. 3. Frog, weight 39 grams, injected with 0.5 c. c. per gram body weight. Ventricle stopped in fifty-seven minutes; auricles beat two hours longer.

TABLE VI.—*Determination of toxic dose for Rana pipiens, Focke method—drug injected into leg lymph sacs*—Continued.

SUMMARY.

Preparation.	V (value).
Mulford No. 1	3.8
N. B. and Co	2.9
B. W. and Co	2.7
H. B. and W	2.7
S. and D	2.4
Lloyd Bros	2.3
P., D. and Co	2.1
Merrell	2.1
Digitalone 1, 2, 3	....

PERFUSION OF FROG'S HEART.

The perfusion experiments upon excised frogs' hearts were carried out by tying a cannula in the left trunk of the aorta and a second in the posterior vena cava near the heart. The isolated heart was perfused under constant pressure with Ringer's solution to which digitalis in tincture strength was added so as to make a 1 per cent solution. The time was noted until systolic stoppage of the heart appeared and the figures recorded indicate the number of minutes the heart continued beating after the introduction of the drug. The results obtained by this method are recorded in Table VII.

Table VII.—*Determination of the time required to stop the isolated heart of Rana pipiens with Ringer's solution to which digitalis had been added to make the solution of 1 per cent tincture strength.*

	Minutes.
Mulford No. 2	23
	26
	21
	21
	18
	22
	14
	15
	15
	16
	21
	18
Average	19
Merrell	31
	35
	17
	35
	37
	34
	21
	35
Average	31
B. W. and Co	19
	30
	29
	21
	26
	17
	18
Average	23
Lloyd Bros	66
	71
	54
	48
	46
Average	57
Digitalone No. 3	53
	47
	78
	65
	97
	72
Average	69

	Minutes.
N. B. and Co	17
	18
	28
	19
	20
	16
	21
Average	20
H. B. and W	17
	15
	21
	23
	16
	18
Average	18
P., D. and Co	20
	32
	12
	33
	44
	26
	12
	16
	19
	22
	26
Average	24
S. and D	29
	27
	25
	32
	26
	32
	29
	20
Average	28

SUMMARY.

Preparation.	Minutes.
H. B. and W	18
Mulford No. 2	19
N. B. and Co	20
B. W. and Co	23
P., D. and Co	24
S. and D	28
Merrell	31
Lloyd Bros	57
Digitalone No. 3	69

THE BLOOD PRESSURE METHOD OF ASSAY.

The blood-pressure method, included in a third group by our classification, was carried out in the ordinary manner, cats being used as experimental animals. They were anaesthetized with chloretone, 0.4 gram per kilogram body weight, dissolved in a small amount of alcohol (2 c. c. to 1 gram chloretone) diluted with a little water and given by the stomach after the method of Edmunds and Cushny.[a] The comparison of the various preparations of digitalis is made upon the percentage of rise in blood pressure which followed the injection of 1 c. c. of the various solutions when diluted with normal salt solution to tincture strength. Only one preparation could be used upon one animal, and from two to four animals were used for each preparation in order that the average of the results obtained might more accurately represent the value of the drug used. The results of these experiments, which are tabulated in Table III, page 37, are summarized in the following table:

Table VIII.—*Summary of results obtained on blood pressure.—Average per cent rise after 1 cubic centimeter doses intravenously.*

Preparation.	Per cent increase.
N. B. and Co	99
Mulford No. 2	64
Digitalone No. 2	55
B. W. and Co	51
P., D. and Co	37
S. and D	35
H. B. and W	33
Merrell	26
Digitalone No. 3	26
Lloyd Bros	24
Digitalone No. 1	[1] −23

[1] Fall.

To enable a comparison to be made of the relative strengths of the different digitalis preparations as obtained by the different methods of assay employed, we have grouped all the findings in Table IX.

Table IX.—*Summary of results obtained by the several methods for the standardization of digitalis preparations.*

LETHAL-DOSE METHODS.

Mice.		Guinea pigs.		Frogs (twelve-hour).		Cats.	
	mg.		*mg.*		*c. c.*		*mg.*
Mulford No. 1	4	B. W. and Co	0.35	B. W. and Co	0.015	B. W. and Co	158
B. W. and Co	4	H. B. and W	.5	Mulford No. 2	.020	P., D. and Co	171
Merrell	6	Merrell	.6	S. and D	.027	N. B. and Co	192
H. B. and W	7	Mulford No. 1	.7	P., D. and Co	.029	Mulford No. 2	195
P., D. and Co	8	P., D. and Co	.7			H. B. and W	219
N. B. and Co	9	N. B. and Co	.9			S. and D	242
S. and D	9	S. and D	.9			Merrell	252
Digitalone No. 1	22	Lloyd Bros	.9			Digitalone No. 3	280
Lloyd Bros	24	Digitalone No. 1	5.0			Lloyd Bros	438
		Digitalone No. 3	[b] 1.5			Digitalone No. 2	550

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[a] Edmunds and Cushny, Laboratory Guide in Experimental Pharmacology, p. 12.

[b] Lived.

Table IX.—*Summary of results obtained by the several methods for the standardization of digitalis preparations*—Continued.

FROG-HEART METHODS.

One hour.		"Focke."		Perfusion.	
	c. c		*V.*		*min.*
Mulford No. 1	0.012	Mulford No. 2	3.8	H. B. and W	18
Mulford No. 2	.016	N. B. and Co	2.9	Mulford No. 2	19
B. W. and Co	.020	B. W. and Co	2.7	N. B. and Co.	20
P., D. and Co.	.021	H. B. and W	2.7	B. W. and Co	23
N. B. and Co	.022	S. and D	2.4	P., D. and Co	24
Merrell	.024	Lloyd Bros	2.3	S. and D	28
H. B. and W	.025	P., D. and Co	2.1	Merrell	31
S. and D	.027	Merrell	2.1	Lloyd Bros	57
Lloyd Bros	.030			Digitalone No. 3	69
Digitalone No. 3	.040				

BLOOD-PRESSURE METHOD.

	Per cent.		*Per cent.*
N. B. and Co	99	H. B. and W	33
Mulford No. 2	64	Merrell	26
Digitalone No. 2	55	Digitalone No. 3	26
B. W. and Co.	51	Lloyd Bros	24
P., D. and Co	37	Digitalone No. 1	[a]–23
S. and D	35		

[a] Fall.

Table X has been arranged from Table IX to show in a numerical way the relative position the preparations experimented with occupy. The figures represent the relative position each preparation holds with reference to its activity as determined by the various methods of assay. In case two preparations showed the same strength (as for instance the Concentrated tincture, B. W. and Co. and Digitol, Mulford on mice) they have been given equal ranking.

Table X.—*Rearrangement of Table IX to show the relative activity of the following preparations as determined by the various methods of assay.*

Preparations.	Lethal dose methods.				Frog heart methods.			Blood Pressure.
	Mice.	Guinea pigs.	Twelve hour.	Cats.	One hour.	"Focke."	Perfusion.	Per cent increase.
B. W. and Co	1	1	([a])	1	2	3	4	4
Mulford	1	4		4	1	1	2	2
Merrell	3	3		7	5	7	7	8
H. B. and W	4	2		5	6	4	1	7
P., D. and Co	5	4		2	3	7	5	5
N. B. and Co	6	6		3	4	2	3	1
S. and D	6	6		6	7	5	6	6
Digitalone No. 1	8	9						
Lloyd Bros	9	8		9	8	6	8	10
Digitalone No. 3		([b])		8	9		9	8
Digitalone No. 2				10				5

[a] The results obtained by the twelve-hour method are not included because too few experiments were carried out according to it to give the preparations their proper rank.

[b] Lived.

DISCUSSION OF METHODS OF ASSAY.

A superficial examination of the tables giving the summary of the results obtained would seem to indicate the practical worthlessness of the biological assay of digitalis preparations by the methods now in vogue. This is especially emphasized by a comparison of Digitol and the Concentrated tincture made by Burroughs, Wellcome & Co. These give comparative values as follows:

	Mice, toxic dose.	Guinea pigs, toxic dose.	Frogs, one hour.
	mgm.	*mgm.*	c. c.
Digitol No. 1	4.0	0.70	0.012
Concentrated tincture	4.0	.35	.020

Here are two preparations which according to the first test are of the same strength; by a second test one is half the strength of the other, and by a third method exactly the reverse relation is found. No explanation can be suggested for the results obtained on mice and guinea pigs; there was no possibility of a mistake, as the results were confirmed on numerous animals as the tables show, and with different solutions made up on different days. A closer examination of the table shows that these results are certainly exceptional, the vast majority being more uniform, as, for example, the two preparations named are shown by most of the methods to be the strongest of any of the specimens examined. Also comparing the four U. S. Pharmacopœia fluid extracts made by Hance Brothers & White, Parke, Davis & Co., Nelson, Baker & Co., and Sharpe & Dohme, these were found by several of the methods to be of very nearly the same strength, differing not more than 25 per cent. Also the Digitalone preparations and Lloyd's Specific Medicine appear at the bottom of the list in almost every table showing by every method their comparative weakness.

There appears thus to be some slight uniformity in the results obtained by the different methods. This is indeed far from close, but this fact is not surprising when the factors concerned in the different methods of assay are considered. In the first place it could hardly be expected that animals differing so widely as cats and frogs, mice and guinea pigs would react exactly alike to the different preparations. The cause of death is not the same in the mice and guinea pigs as in the frogs and the cats under the conditions of our experiments. With frogs the action is always one upon the heart; also in the cats we maintained artificial respiration so that the figures obtained there indicate a cardiac action. On both mice and guinea pigs, however, the cause of death in probably every case is not due to an action upon the heart but upon the medulla. In every animal we examined we found the heart beating after the respiration had stopped, and it continued to beat as long as artificial respiration was maintained. It would not then be at all surprising if two solutions which showed a certain relationship when the cardiac action was concerned should show an entirely different relation when the central nervous system was the point of attack. One solution might be very weak in its action upon the heart and yet contain decomposition products of digitalis whose typical action is upon the medulla and it would therefore appear unduly strong when judged by such a standard. For this reason *we think that methods which employ as a standard the minimum lethal dose obtained upon the higher animals are not applicable to the physiological assay of the digitalis series.*

It is possible that still further complications would arise in using such methods in that on certain animals of the same species some would die from the cardiac effect and others from the medullary action. In some of the cats, for instance, distinct respiratory movements were seen after the heart had stopped and the blood pressure had fallen to zero; in others the respiration stopped first. As stated earlier, to avoid complications of this sort, in our experiments on cats we always employed artificial respiration.

Comparing the toxic doses obtained upon cats and frogs (twelve-hour method) we have in both cases a cardiac action and three of the four preparations show relatively the same activity; the Parke, Davis & Co. Fluid Extract alone being an exception. But that an exception should occur is not to be wondered at when the differences between the heart of the frog and that of the cat are considered. Also in the blood pressure experiments on the cat we have to do with substances acting upon the vessel walls as well as upon the heart and this necessarily introduces another factor as some of the digitalis active principles act more strongly upon vessel walls than others, and in two preparations of digitalis the active substances may not be present in the same relative amounts.

The "Focke" and the "one-hour" methods upon frogs might be thought to give a closer agreement, but here (aside from the difficulties connected with Focke's method which have been discussed) we have to deal with the question of ease of absorption. As has been pointed out by some earlier writers, in those methods in which the time element is very limited, a weak preparation with easily absorbable constituents would appear stronger than a strong preparation with constituents which are absorbed with difficulty. It would seem that such an objection might be of importance in the "Focke" method, where from seven to twenty minutes only are allowed. As to whether it would play a part in the "one-hour" method is of course possible but not very probable, when it is remembered with what rapidity the action appears when digitoxin in solution is injected.

The reason that the "frog's heart profusion" method does not agree with the other frog methods is possibly due partly to the more uniform character of the solution of the poison in which the heart is bathed and also to the resinous precipitate which forms and falls to the bottom of the perfusion vessel and which no doubt contains some of the active constituents.

From this short critical review of the different methods for standardization it can easily be seen why no two methods of assay could be expected to give exactly the same relative values to a series of digitalis preparations, especially when they are made according to radically different methods, such differences as exist, for instance, between a "Fat free" tincture and a "Specific medicine." The agreement is much closer when the preparations are made according to the same formula as we have in the case of the official fluid extracts.

It would appear then most desirable if manufacturing houses could agree upon a certain method to be used which would insure the different preparations upon the market under the same name having the same potency. As to the choice of such a method difficulties would arise, but certainly none which would appear insurmountable. The method chosen should be as simple as possible, and above all *an action upon the circulation should be taken as a standard of comparison rather than an action upon the nervous system.* Judged by these rules, some of the methods now in use are not suitable for the purpose.

First, we have the toxic action upon the higher animals, which should not be used for the reasons given earlier. The technical difficulties of the Focke method, together with the question of completeness of absorption, exclude it. The perfusion of the frog's heart is also excluded for reasons mentioned and also because it requires more skill to carry it out, and even then the variations in results are very great. The blood-pressure method upon cats and dogs commends itself on account of the close relation it sustains to the use of the drug in clinical practice. The objections consist in the difficulty of procuring these animals at times and also the necessity of carrying out repeated experiments to confirm the results, which a study of our tables show will vary very greatly. A dose of a certain preparation which in one animal may cause a rise of perhaps 70 or 80 per cent in blood pressure may in others not raise it more than one-quarter as much, but probably repeated tests will give reliable results.

The toxic dose can also be obtained at the same time by using artificial respiration, but, as our tables show, there need be no agreement between the two, and it therefore seems superfluous. This leaves, then, the two frog methods—the "twelve-hour" toxic method and the "one-hour" method. As between them it is impossible to choose with any degree of accuracy. With the "one-hour" method we have the advantage of there being no delay, it being possible to assay a preparation in three or four hours, and it probably takes fewer frogs than the "twelve-hour" method. On the other hand, with the latter method, although taking four or five days to complete the assay, it need not interfere with other work, as the frogs can be injected late in the afternoon and examined the next morning. Between these two methods, then, as far as can be judged, it is largely a question of personal preference and convenience, at least in the light of our present knowledge. Our results do not show that they give the same relative values to the four specimens examined by them, and as to which is correct it is impossible to say. They do roughly agree in that Burroughs, Wellcome & Co.'s Concentrated Tincture and Digitol are both stronger than the two fluid extracts of Parke, Davis & Co. and Sharpe & Dohme. This relationship is confirmed by every other method which we employed, excepting that using the toxic dose as found on cats. It would not seem, therefore, to be unreasonable to suppose that such a relationship would probably exist in man.

As to the desirability of manufacturing houses standardizing their preparations belonging to the digitalis series there can be no question.

It is true that the official preparations examined herewith are approximately of the same strength, but the crude drug obtained in another year may represent altogether a different toxicity to that employed in the manufacture of these preparations, and the only way at present to avoid like variations in the tinctures and fluid extracts is to standardize them by physiological methods. It is not claimed that such methods are infallible. They certainly are not, as we have shown; but, as we have also shown, most of the apparent discrepancies are due to the fact that actions upon quite different physiological functions are taken as a measure of activity. Such discrepancies can be avoided by the adoption of one or the other of these frog methods, and if it is thought necessary the results obtained can be confirmed by blood-pressure experiments upon cats or, preferably, dogs.

We believe the chief point to be kept in mind is that, in spite of annoying individual discrepancies, there is a general agreement between the various methods. This agreement, too, is much more complete in the case of those methods in which the toxic effect is due to an action upon the heart. A preparation found weak by one method appears weak by all methods, and one showing marked activity by one shows the same result in all.

The second part of the research, that which is concerned with the different preparations, requires little to be added to that already given in the tables. We have arranged, however, the different preparations in the order of their relative strengths as determined by the results obtained by all the methods of assay. Such a list is found in Table XI. This is prepared from Table X by adding together the numbers denoting the relative position of each preparation and dividing the sum by the number of assay methods used. Naturally the quotients thus found will stand in inverse ratio with the strength of the preparation.

In this table the results obtained by the "twelve-hour" method on frogs were not used, as only four preparations were assayed according to it, so that no true ranking in relation to the results obtained by the other methods could be found.

TABLE XI.

Preparation.	Sum of ratings.	Number of assays.	Final result.
Mulford	15	7	2.14
B., W. and Co	16	7	2.28
N., B. and Co	25	7	3.57
H. B. and W	29	7	4.14
P., D. and Co	31	7	4.43
Merrell	40	7	5.71
S. and D	42	7	6.00
Lloyd Bros	58	7	8.28
Digitalone No. 3	34	4	8.50
Digitalone Nos. 1 and 2	....	....	....

Such a table as this we think, on the whole, represents very fairly the relative strengths of the different preparations examined. It represents the final conclusion drawn from a very large series of experiments, and on that account may be considered fairly accurate.

There are only four preparations which would appear to require special mention. These are the normal tincture of digitalis made by William S. Merrell Chemical Company, of Cincinnati; digitalone made by Parke, Davis & Co., of Detroit; the specific medicine, digitalis made by Lloyd Bros., of Cincinnati, and tincture digitol made by H. K. Mulford & Co., of Philadelphia. Two bottles of digitol were examined. By the one-hour method on frogs they did not show the same strength, one requiring 0.012 c. c. to produce systolic stoppage of the heart in one hour and the other 0.016 c. c. (Table IX.) This preparation, as mentioned earlier, is "assayed, tested physiologically" and standardized to contain 0.025 gram digitoxin in 100 c. c.

The purified normal tincture of Merrell is said to be "a standardized neutral tincture of prime digitalis leaves freed from the irritating fats and oils." In our tables the dose of this preparation was always calculated upon the basis of fluid extract strength, which is not correct, as normal tinctures are said to be made from fresh leaves which are put into alcohol. Later the alcohol is expressed and by evaporation 1 kilo of the solution is made equivalent to 1 kilo of leaves. It is thus considerably weaker than a fluid extract on account of the moisture content of the fresh plant. But even calculated in this way it compares fairly well with the fluid extracts, especially by the "toxic" methods. The maximum dose recommended is about 3 minims, or about the same as for a fluid extract.

The "Specific Medicine" digitalis, made by Lloyd Bros., of Cincinnati, offered some difficulties on account of its high alcohol content (80 per cent absolute). If this was evaporated off, a gummy resinous mass was left which was very hard to mix with water in such a way as to give uniform dosage. In many cases to avoid this trouble the solution was merely exposed in any open vessel at room temperature until part of the alcohol had evaporated, and the residue was then mixed with the water to get the desired dilution. The solutions thus obtained were always thoroughly shaken to insure a uniform distribution of the precipitate. In one method, namely, the perfusion of the frog's heart, it is not possible to get the full action of the drug, as the precipitate which forms when the drug is added to the Ringer's solution settles to the bottom of the bottle containing the perfusion fluid and only the soluble portion reaches the heart. It is for this reason that the drug appears so much weaker by this method than by the other methods in which the animal receives the entire drug. The results of our experiments show that the preparation is uniformly weaker than the pharmacopœial fluid extracts. According to the label its strength is 480 grains to the fluid ounce.

In the advertising literature of this firm the effect upon the system of the "Specific Medicines" is said to be about double that of ordinary fluid extracts and the dose should not be more than one-half the usual dose of fluid extracts. The dose recommended on the bottle is from one-third minim to 1 minim *every hour*, which is certainly not less than that of a pharmacopœial fluid extract. In general, its strength would appear to be about the same as the fluid extracts; it certainly is not stronger.

Three bottles of digitalone were examined. This is said to be an aseptic nonalcoholic, permanent solution of digitalis of the same strength as the U. S. Pharmacopœia tincture. Bottles Nos. 1 and 2 were not essentially different in the character of the preparation contained. In both cases it possessed a peculiar, somewhat aromatic odor, not exactly unpleasant, but somewhat sour. The fluids were both a dark brown and contained a rather heavy precipitate. Bottle No. 3, the odor was not as unpleasant, and somewhat suggested chloretone. The preparation was slightly lighter in color than an orange yellow, and contained a fairly abundant, fine white, flaky precipitate.

Their relative physiological activity can be most clearly illustrated by three experiments upon frogs carried out under similar conditions, the hearts being exposed and the drug being injected in relatively the same size doses.

Digitalone No. 1.

August 17, 1908. Frog, 41 grams:
11.25. Heart rate 27 in twenty seconds.
11.29. Heart rate 28 in twenty seconds.
11.30. Injected 2.05 c. c. digitalone No. 1; 0.05 c. c. per gram body weight.
11.42. Heart rate 18 in twenty seconds.
11.50. Heart rate 16 in twenty seconds.
12.01. Heart rate 16 in twenty seconds.
12.30. Heart rate 17 in twenty seconds.
2.15. Heart rate 16 in twenty seconds; heart weak.
4.30. Heart rate 16 in twenty seconds; dilates poorly and very weak contraction.

Digitalone No. 2.

August 17, 1908. Frog, 40 grams:
11.00. Heart rate 30 in twenty seconds.
11.05. Heart rate 37 in twenty seconds.
11.05. Injected 2 c. c. digitalone No. 2.
11.10. Heart rate 15 (?) in thirty seconds; irregular peristaltic contractions.
11.18. Heart rate 16 in twenty seconds; normal contractions.
11.27. Heart rate 16 in twenty seconds.
11.41. Heart rate 16 in twenty seconds; systole weak.
11.50. Heart rate 16 in twenty seconds.
12.30. Heart rate 15 in twenty seconds.
2.15. Heart rate 17 in twenty seconds; weak.
4.15. Heart rate 17 in twenty seconds; weak, dilating fairly well.

Digitalone No. 3.

August 17, 1908. Frog, 39 grams:
10.55. Heart rate 26 in twenty seconds.
11.01. Heart rate 26 in twenty seconds.
11.02. Injected 1.95 c. c. digitalone No. 3.
11.06. Heart rate 11 in twenty seconds; systole prolonged.
11.12. Heart rate 10 in twenty seconds.
11.16. Heart rate 16 in twenty seconds; dilates little.
11.40. Heart rate 7 in twenty seconds, little dilatation.
11.59. Ventricle in systole; auricles, rate 7 in twenty seconds.
2.30. Auricle stopped; ventricle in systole.

Specimens Nos. 1 and 2 showed no digitalis action whatever, while No. 3 was quite weak.

To kill mice Digitalone No. 1 required from two and one-half to five times the dose that any other preparation examined required excepting Lloyd's "Specific Medicine, digitalis." On guinea pigs 2 milligrams per kilogram body weight caused death, but as pointed out earlier it was a question whether this was due to the digitalis per se or to the effect of an injection under the skin of 12.5 c. c. of fluid containing chloretone and possibly digitalis decomposition products. The animal became comatose at once, showing no characteristic digitalis symptoms. Preparation No. 2, tested by the twelve-hour method on frogs, did not cause death in doses as high as 0.07 c. c. per gram body weight, while the preparations made according to the other methods caused death in doses from one-quarter to one-half as large. In the one-hour method Digitalone No. 2, in doses four times as large as were given of the weakest and ten times as large as the strongest of the other (excepting Digitalone) preparations, failed to call forth systolic standstill of the heart. Digitalone No. 3 seemed to be fairly active. By the one-hour method on frogs it was from one-third to two-thirds as strong as the other preparations. Focke's method was not very successful on account of the extremely large dose which would have required so much concentration on the water bath to bring it to a volume small enough to inject into the lymph sacs. This specimen failed to kill guinea pigs when used in doses twice as large as the average preparation required. Injected into rabbits Digitalone No. 1, instead of raising the blood pressure, lowered it 23 per cent, the curve resembling that given by the nitrite series. Digitalone No. 3 raised the blood pressure 26 per cent.

An interesting fact in connection with the value of the blood-pressure methods of assay is that Digitalone No. 2, which was shown by the exposed frog's heart to be almost devoid of digitalis action, caused a rise of 55 per cent in the blood pressure of two cats. It might be that some other substances are present (perhaps decomposition products) which, possessing no cardiac effect, yet act on the vasomotor system, probably upon the center. In specimen No. 1 other changes in the solution must have occurred, as at each injection a fall in blood pressure followed. The important conclusion to be drawn from these experiments is that Digitalone is not invariably a permanent solution. Those preparations which have decomposed are not only devoid of any digitalis action, but are distinctly harmful. A preparation which evidently had not deteriorated completely seemed to be about half the strength of an official tincture.

LIST OF HYGIENIC LABORATORY BULLETINS OF THE PUBLIC HEALTH AND MARINE-HOSPITAL SERVICE.

The Hygienic Laboratory was established in New York, at the Marine Hospital on Staten Island, August, 1887. It was transferred to Washington, with quarters in the Butler Building, June 11, 1891, and a new laboratory building, located in Washington, was authorized by act of Congress, March 3, 1901.

The following *bulletins* [Bulls. Nos. 1-7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.] have been issued:

*No. 1.—Preliminary note on the viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau.

*No. 3.—Sulphur dioxid as a germicidal agent. By H. D. Geddings.

*No. 4.—Viability of the *Bacillus pestis*. By M. J. Rosenau.

No. 5.—An investigation of a pathogenic microbe (*B. typhi murium* Danyz) applied to the destruction of rats. By M. J. Rosenau.

*No. 6.—Disinfection against mosquitoes with formaldehyd and sulphur dioxid. By M. J. Rosenau.

No. 7.—Laboratory technique: Ring test for indol, by S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis; Microphotography with simple apparatus, by H. B. Parker.

By act of Congress approved July 1, 1902, the name of the "United States Marine-Hospital Service" was changed to the "Public Health and Marine-Hospital Service of the United States," and three new divisions were added to the Hygienic Laboratory.

Since the change of name of the Service the bulletins of the Hygienic Laboratory have been continued in the same numerical order, as follows:

*No. 8.—Laboratory course in pathology and bacteriology. By M. J. Rosenau. (Revised edition, March, 1904.)

*No. 9.—Presence of tetanus in commercial gelatin. By John F. Anderson.

No. 10.—Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States. By Ch. Wardell Stiles.

*No. 11.—An experimental investigation of *Trypanosoma lewisi*. By Edward Francis.

*No. 12.—The bacteriological impurities of vaccine virus; an experimental study. By M. J. Rosenau.

*No. 13.—A statistical study of the intestinal parasites of 500 white male patients at the United States Government Hospital for the Insane; by Philip E. Garrison, Brayton H. Ransom, and Earle C. Stevenson. A parasitic roundworm (*Agamomermis culicis* n. g., n. sp.) in American mosquitoes (*Culex sollicitans*); by Ch. Wardell Stiles. The type species of the cestode genus *Hymenolepis;* by Ch. Wardell Stiles.

No. 14.—Spotted fever (tick fever) of the Rocky Mountains; a new disease. By John F. Anderson.

No. 15.—Inefficiency of ferrous sulphate as an antiseptic and germicide. By Allan J. McLaughlin.

*No. 16.—The antiseptic and germicidal properties of glycerin. By M. J. Rosenau.

*No. 17.—Illustrated key to the trematode parasites of man. By Ch. Wardell Stiles.

*No. 18.—An account of the tapeworms of the genus *Hymenolepis* parasitic in man, including reports of several new cases of the dwarf tapeworm (*H. nana*) in the United States. By Brayton H. Ransom.

*No. 19.—A method for inoculating animals with precise amounts. By M. J. Rosenau.

*No. 20.—A zoological investigation into the cause, transmission, and source of Rocky Mountain "spotted fever." By Ch. Wardell Stiles.

No. 21.—The immunity unit for standardizing diphtheria antitoxin (based on Ehrlich's normal serum). Official standard prepared under the act approved July 1, 1902. By M. J. Rosenau.

*No. 22.—Chloride of zinc as a deodorant, antiseptic, and germicide. By T. B. McClintic.

*No. 23.—Changes in the Pharmacopœia of the United States of America. Eighth Decennial Revision. By Reid Hunt and Murray Galt Motter.

No. 24.—The International Code of Zoological Nomenclature as applied to medicine. By Ch. Wardell Stiles.

No. 25.—Illustrated key to the cestode parasites of man. By Ch. Wardell Stiles.

No. 26.—On the stability of the oxidases and their conduct toward various reagents. The conduct of phenolphthalein in the animal organism. A test for saccharin, and a simple method of distinguishing between cumarin and vanillin. The toxicity of ozone and other oxidizing agents to lipase. The influence of chemical constitution on the lipolytic hydrolysis of ethereal salts. By J. H. Kastle.

No. 27.—The limitations of formaldehyde gas as a disinfectant with special reference to car sanitation. By Thomas B. McClintic.

*No. 28.—A statistical study of the prevalence of intestinal worms in man. By Ch. Wardell Stiles and Philip E. Garrison.

*No. 29.—A study of the cause of sudden death following the injection of horse serum. By M. J. Rosenau and John F. Anderson.

No. 30.—I. Maternal transmission of immunity to diphtheria toxine. II. Maternal transmission of immunity to diphtheria toxine and hypersusceptibility to horse serum in the same animal. By John F. Anderson.

No. 31.—Variations in the peroxidase activity of the blood in health and disease. By Joseph H. Kastle and Harold L. Amoss.

No. 32.—A stomach lesion in guinea pigs caused by diphtheria toxine and its bearing upon experimental gastric ulcer. By M. J. Rosenau and John F. Anderson.

No. 33.—Studies in experimental alcoholism. By Reid Hunt.

No. 34.—I. *Agamofilaria georgiana* n. sp., an apparently new roundworm parasite from the ankle of a negress. II. The zoological characters of the roundworm genus *Filaria* Mueller, 1787. III. Three new American cases of infection of man with horsehair worms (species Paragordius varius), with summary of all cases reported to date. By Ch. Wardell Stiles.

*No. 35.—Report on the origin and prevalence of typhoid fever in the District of Columbia. By M. J. Rosenau, L. L. Lumsden, and Joseph H. Kastle. (Including articles contributed by Ch. Wardell Stiles, Joseph Goldberger, and A. M. Stimson.)

No. 36.—Further studies upon hypersusceptibility and immunity. By M. J. Rosenau and John F. Anderson.

No. 37.—Index-catalogue of medical and veterinary zoology. Subjects: Trematoda and trematode diseases. By Ch. Wardell Stiles and Albert Hassall.

No. 38.—The influence of antitoxin upon post-diphtheritic paralysis. By. M. J. Rosenau and John F. Anderson.

No. 39.—The antiseptic and germicidal properties of solutions of formaldehyde and their action upon toxines. By John F. Anderson.

No. 40.—1. The occurrence of a proliferating cestode larva (*Sparganum proliferum*) in man in Florida, by Ch. Wardell Stiles. 2. A reexamination of the type specimen of *Filaria restiformis* Leidy, 1880=*Agamomermis restiformis*, by Ch. Wardell Stiles. 3. Observations on two new parasitic trematode worms: *Homalogaster philippinensis*

n. sp., *Agamodistomum nanus* n. sp., by Ch. Wardell Stiles and Joseph Goldberger. 4. A reexamination of the original specimen of *Tænia saginata abietina* (Weinland, 1858), by Ch. Wardell Stiles and Joseph Goldberger.

*No. 41.—Milk and its relation to the public health. By various authors.

No. 42.—The thermal death points of pathogenic micro-organisms in milk. By M. J. Rosenau.

No. 43.—The standardization of tetanus antitoxin (an American unit established under authority of the act of July 1, 1902). By M. J. Rosenau and John F. Anderson.

No. 44.—Report No. 2 on the origin and prevalence of typhoid fever in the District of Columbia, 1907. By M. J. Rosenau, L. L. Lumsden, and Joseph H. Kastle.

No. 45.—Further studies upon anaphylaxis. By M. J. Rosenau and John F. Anderson.

No. 46.—*Hepatozoon perniciosum* (n. g. n. sp.); a haemogregarine pathogenic for white rats; with a description of the sexual cycle in the intermediate host, a mite (*Lelaps echidninus*). By W. W. Miller.

No. 47.—Studies on Thyroid.—I. The Relation of Iodine to the Physiological Activity of Thyroid Preparations. By Reid Hunt and Atherton Seidell.

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